THE UNIVERSITY OF ALBERTA

THE TAXONOMY OF *VIOLA ADUNCA* J.E. SMITH

by

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A THESIS

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The undersigned certify that they have read, and that they recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "THE TAXONOMY OF VIOLA ADUNCA J.E. SMITH" submitted by Gordon D. McPherson in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The diploid and tetraploid chromosome races of Viola adunca J.E. Smith are compared taxonomically. The systematic position of V. adunca is outlined, its synonymy is given, and a review of the literature is presented. Investigations were carried out on the morphology, chromatographic properties, cytology, and reproductive behavior of the two races in an attempt to clarify the taxonomic status of the tetraploid. Some macroscopic morphological differences exist between the two polyploid levels. Microscopic features of cell size provide an improved basis for recognition of chromosomal level. Paper and thin-layer chromatography indicate that few chemical differences exist, although each Viola species examined displays a unique chromatographic pattern. The distribution of the tetraploid is more northern than that of the diploid, at least in the western interior of North America, and an attempt is made to explain this allopatry in terms of the geological history and current climatic conditions of this area. The sterility of triploid hybrids indicates that the diploid and tetraploid are reproductively isolated. On the basis of these results, it is concluded that the tetraploid is an autopolyploid, and that it should be recognized as a distinct subspecies.

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TABLE OF CONTENTS

	Page
ABSTRACT	i
ACKNOWLEDGEMENTS	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	v
LIST OF ILLUSTRATIONS	vi
INTRODUCTION	1
Description and taxonomy of the genus	3
Evolutionary history of the genus	7
Biosystematic studies in *Viola*	8
Description and synonymy of *Viola adunca*	15
Cytology and phytogeography of *Viola adunca*	19
MATERIALS AND METHODS	23
Specimens	23
Collecting techniques	23
Cultivation	24
Guard cell and pollen measurements	25
Cytology	25
Bagging	26
Chromatographic techniques	26
TAXONOMY	31
Morphology	31
Guard cells	37
Pollen size	37
Cytology	40
Bagging	45

Page

Paper chromatography 45

Thin layer chromatography 52

Distribution 54

Dispersal 68

Interracial breeding 69

Conclusion 73

REFERENCES 77

LIST OF TABLES

Table		Page
I.	Sections of *Viola*.	5
II.	Subsections of section *Viola*.	6
III.	Some polyploid series found in North American *Viola*.	11
IV.	Chromosome counts of *Viola adunca*.	20
V.	Solvent systems used in chromatography.	29
VI.	Cytologically examined specimens.	42
VII.	Chromatographic results.	47

LIST OF ILLUSTRATIONS

Plate		Page
I.	*Viola adunca*.	viii
II.	Style apices in *Viola adunca*.	35
III.	Lacto-phenol staining properties of diploid, triploid, and tetraploid specimens.	71

Figure		Page
1.	Distribution of the genus *Viola* in the northern hemisphere.	2
2.	Relationships of northern hemisphere violets.	9
3.	Distribution of *Viola adunca*.	17
4.	Stipule length frequencies in the diploid and tetraploid.	32
5.	Sepal length frequencies in the diploid and tetraploid.	33
6.	Ranges of variation in protuberances of stylar apices in the diploid and tetraploid races of *V. adunca*.	36
7.	Guard cell length frequencies in the two chromosome races.	38
8.	Pollen size in the two chromosome races.	39
9.	Somatic chromosomes of the two chromosome races.	41
10.	Metaphase I in the tetraploid.	41
11.	Generalized paper chromatograph.	49
12.	Paper chromatographic patterns of *Viola rugulosa* and *V. selkirkii*.	50

Figure Page

13. Paper chromatographic patterns of *Viola palustris* and *V. nephrophila*. 51

14. Thin layer chromatographic pattern of *Viola adunca*. 53

15. Collection sites in western Canada. 55

16. Distribution of chromosome races in North America. 57

17. Maximum extent of the Wisconsin glaciation. 60

18. Soil types of the Prairies. 64

19. Spring isotherms of the Prairies. 66

20. Number of days above 60° F. 67

Plate I. *Viola adunca*

INTRODUCTION

The genus Viola L. is a member of the Violaceae De Candolle (in Lamarack and De Candolle, Flora Francaise 4: 801. 1805). The family is largely tropical and subtropical. The single genus Viola has its main area of distribution within the temperate belts, and in the mountains of the tropics (Fig. I). The species of all the genera except Viola are arborescent or shrubby, while those of Viola include also many perennial and annual herbs. Actinomorphy is the rule within the family, and Viola is once again an exception. At both the generic level and the familial level the morphologically primitive groups are found in the tropical regions of Central and South America. Added to this, 14 of the 16 genera in the family occur there, and consequently this area is believed by some (Clausen 1929) to have been the place of origin of the family, and of the genus Viola.

Melchior (1925) recognizes two subfamilies--the Violoideae and the Leonoideae, the latter containing one genus, Leonia Ruiz. and Pav. He lists two tribes under the Violoideae--the Rinoreeae Reiche and Taubert and the Violeae Gingins, and in the latter tribe are recognized the Hybanthinae, and the Violinae. In this last group he places the genera Anchietea St. Hil., Corynostylis Mart., Schweiggeria Sprengel, Noisettia H.B. and K., and Viola L.

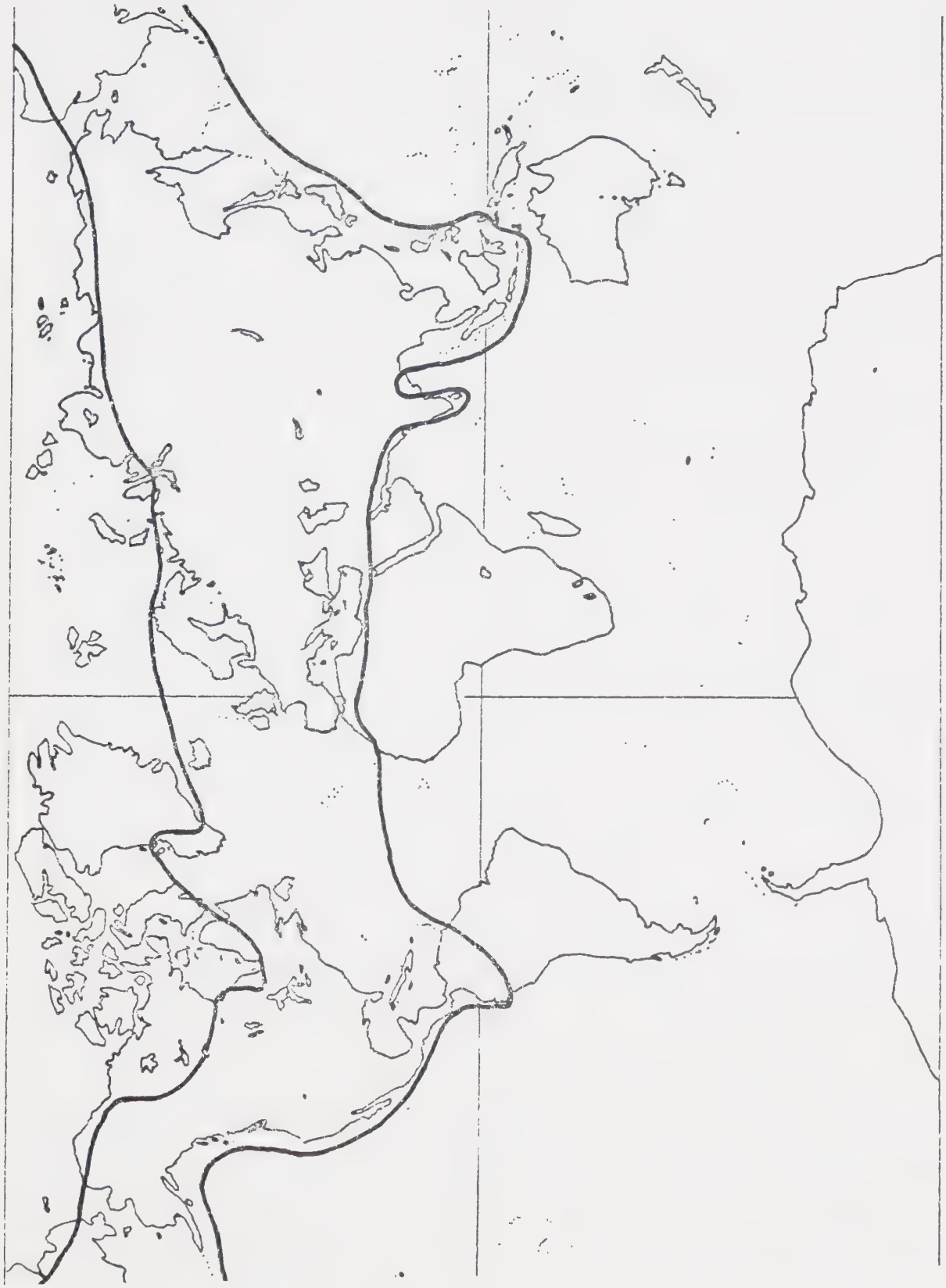


Fig. 1. Distribution of the genus Viola in the northern hemisphere. (after Clausen 1929)

Description and taxonomy of the genus.

Viola Linnaeus, Sp. Plant. Ed. I. 933.1753.

Small trees, shrubs of herbs, with a somewhat irregular 1-spurred or gibbous corolla of 5 petals, 5 hypogynous stamens with adnate introrse anthers connivent over the pistil, and a 1-locular 3-valved capsule with 3 parietal placentae. Sepals 5, persistent, auricled. Petals imbricated in the bud, the lower one spurred at the base. Stamens with their short and broad filaments continued beyond the anther-locules, often coherent with each other, and closely surrounding the ovary; the two lower ones bearing spurs which project into the spur of the corolla. Style usually clavate, with the simple stigma turned to one side. Valves of the capsule bearing the several-seeded placentae on their middle; after opening each valve, as it dries, folding firmly together lengthwise, projecting the seeds. Seeds anatropous, with a hard seed-coat, and a large straight embryo nearly as long as the albumen; cotyledons flat. Leaves alternate, with stipules. Flowers axillary, nodding; cleistogamous flowers produced in most sections. (Fernald 1950).

A necessary adjunct to a description of the genus is a consideration of the peculiar type of self-pollinating, reduced flower which ensures the production of seeds in case the colourful, outcrossing flowers should go unpollinated. These flowers, termed cleistogamous, are found in most species of the genus. Madge (1929) describes the structure of a typical flower. The calyx remains in a folded position and encloses within it the rudiments of the petals, the androecium (which in some species is reduced to two or three stamens, none of which are spurred), and the pistil, the style of

which is usually bent towards the stamens. The opening of the style is generally expanded and faces the pollen sacs, and in some species the style opening is appressed to the top of one or two anthers. The pollen germinates *in situ* and grows out to the stylar opening and the ovary. Thus there have been several evolutionary modifications towards reduction and adaptation. Faegri and van der Pijl (1966) consider cleistogamic flowers like those of *Viola* to be the final stages in a recognizable series of autogamy that begins with a breakdown of external and internal factors preventing self-fertilization. In *V. adunca* the cleistogamous flowers appear in summer following the production of the chasmogamous (open) flowers during the spring flowering.

The over-300 species in the genus are usually placed in the fourteen sections accepted by Becker (1925), only three of which are found in North America (see Table I).

Table I. Sections of *Viola*.

Section	Distribution
*Viola**	Northern Hemisphere
Dischidium Ging.	Southeast Asia
Chamaemelanium Ging.	Northern Hemisphere
Melanium Ging.	Europe (1 species in North America)
Xylinosium W. Bckr.	Europe
Delphiniopsis W. Bckr.	Mediterranean area
Sclerosium W. Bckr.	Africa
Leptidium Ging.	South America
Nosphinium W Bckr.	Hawaii
Rubellium W. Bckr.	Chile
Andinium W. Bckr.	South America
Chilenium W. Bckr.	South America
Tridens W. Bckr.	South America
Erpetion (Sweet) W. Bckr.	Australia

*This section, called *Nomimium* Ging. by Becker, contains *V. odorata* L., the type species (as designated by Britton and Brown in 1913), and is therefore properly named the section *Viola* (Article 22 of the International Code of Botanical Nomenclature).

Of the three North American sections only Viola is of interest here. Becker (1925), whose classification is the most widely accepted, divided it into seventeen subsections (Table II), based mainly on characters of leaf shape, rhizome, flower colour and habit.

Table II. Subsections of section Viola.

Subsection	Distribution
Uncinatae Kupffer	Eurasia
Rostratae Kupffer	Northern Hemisphere
Repentes Kupffer	Europe
Lignosae W. Bckr.	Near East
Memorabiles W. Bckr.	East Asia
Stolonosae Kupffer	Western Hemisphere
Adnatae W. Bckr.	Northern Hemisphere
Vaginatae W. Bckr.	Asia
Langsdorffianae W. Bckr.	Northern Pacific Basin
Serpentes W. Bckr.	Asia
Diffusae W. Bckr.	Asia
Bilobatae W. Bckr.	Asia
Boreali-Americanae W. Bckr.	North America
Pedatae Pollard	Eastern North America
Orbiculares Pollard	Eastern North America
Mexicanae W. Bckr.	South and Central America
Umbraticolae W. Bckr.	Central America

Clausen (1929) has proposed a modified classification, uniting the Boreali-Americanae, Adnatae and Stolonosae in the

section Plagiostigma, but his system has not been accepted generally as any improvement on that of Becker (Valentine 1962). The Rostratae are split into three series--the Scapigerae W. Bckr., the Arosulatae Borb. and the Rosulantes Borb. The Scapigerae are Eurasian plants and are usually without stems, although sometimes there are stems functioning as runners; the Arosulatae are Eurasian plants of little apparent differentiation; the circumpolar Rosulantes of which Viola adunca is a member are plants in which the main stem is reduced, and in which the flowers develop on side stems from axils of the leaves in the rosette.

Evolutionary history of the genus.

Because of its frequently herbaceous habit, its zygomorphy, and its primarily temperate distribution, the genus Viola is regarded as the peak of evolution within the family (Clausen 1951). It has been suggested (Camp 1947) that some species of the genus became pre-adapted to the temperate regions of the Western Hemisphere via an evolution of forms which were adapted to the foothills and mountains of tropical America. Invasion of the temperate regions of the Western Hemisphere then occurred along the Cordilleran mountains. Something of the success of the genus in radiating to fit a wide variety of ecological niches is reflected in the fact that over 300 species are recognized by conservative taxonomists (Becker, 1925).

The Northern Hemisphere violets are thought (Clausen 1929) to have evolved from the Chamaemelanium section, a group centered in Mexico and along the Pacific coast of North America. Within it are some species which resemble members of the section Viola, and others

which resemble members of the section Melanium. Clausen (1929) presents this view of the North American groups, based largely on study of the style and leaf shapes (Fig. 2).

A morphological series can be drawn for the style heads showing a progression from those of the Chamaemelanium section to those of the Rostratae. This sequence involves trends from cleft heads with large, unprotruded openings to heads that are covered, smaller, and with the opening protruded on a rostellum (Clausen 1929).

Crossing experiments (Gershoy 1928) indicate that the Rosulantes is the central or primitive group of the Rostratae, for successful crosses have been made between species of Rosulantes and species of Scapigerae and of Arosulatae (and also of the subsections Boreali-Americanae, Adnatae and Stolonosae, and of the section Chamaemelanium) but crosses between the Arosulatae and the Scapigerae are unsuccessful. Also, the Rosulantes are found in North America (the group is circumpolar) while the Arosulatae are Eurasian, and the Scapigerae Mediterranean and West Asiatic. Thus the Rosulantes may have been the ancestral group from which the Scapigerae and Arosulatae separately diverged.

Biosystematic studies in Viola.

The genus has been the object of some of the more modern techniques of inquiry. Genetic and cytological work, hybridization studies and a small amount of chemical investigation have all been carried out in the genus of segments of it.

Clausen found in Viola species valuable material for the

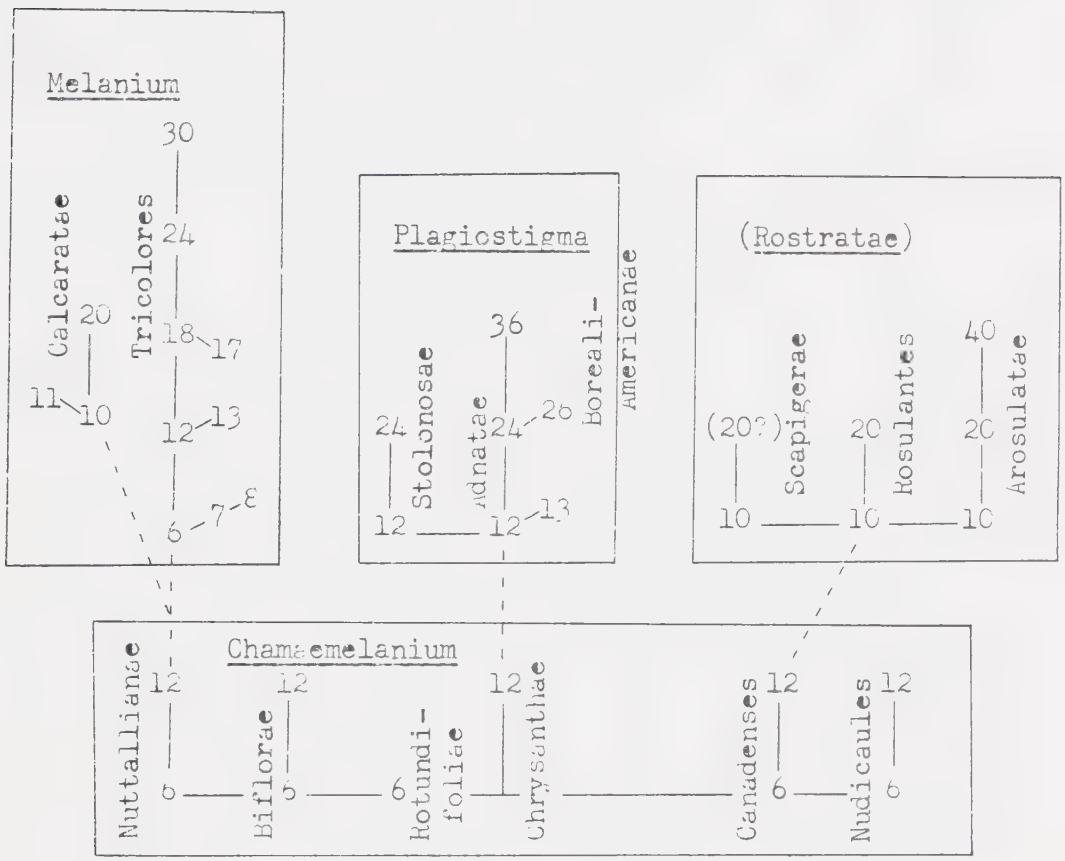


Fig. 2. Relationships of northern hemisphere violets.

(numbers refer to haploid chromosome complement)

(modified after Clausen 1929)

study of racial differentiation. For example, V. tricolor L. (Clausen 1921, 1922) was discovered to have at least five races marked by differences in leaf size and outline, habit, and life cycle, depending upon the habitat from which they were collected. The genetic nature of ecological racial adaptations was also demonstrated using this species for study (Clauser, Keck & Hiesey 1940, Clausen 1951). Studies on introgression between species (Russell 1955, Russell and Cooperrider 1955) have been undertaken using the method of "extrapolated correllates" associated with Anderson's name (1929).

Gershoy (1928), Miyaji (1913), Heilborn (1926), and Clausen (1929), demonstrated that each section of the genus has a different basic chromosome number, and the systematic position of certain species and larger groupings has been considerably clarified by a cytological examination. For example, the observation that the section Chamaemelanium has a basic number x=6 supports the view that it is the section from which the North American groups have arisen, for in these taxa the known basic numbers are x=10 (Rostratae, or x=11 or 12 (Stolonosae, Adnatae), or x=26 or 27 (Boreali-Americanae, Pedatae).

Several examples of polyploid series can be found in each of the North American subsections that have been examined (Table III) (Muntzing 1936).

Table III. Some polyploid series found in North American Viola.

Subsection	Chromosome number (n)	Representative species
Rostratae		
Rosulantes	10	V. mirabilis L., V. adunca
	20	V. riviniana Rchb.
Arosulatae	10	V. stagnina Kit.
	20	V. elatior Fries.
	40	V. canina L.
Adnatae	12	V. selkirkii Goldie
	24	V. pinnata L.
	36	V. patrini Ging.

The species that stand in a polyploidal relation to one another are distinguishable to various extents from one another. The gradient runs from species in which no division is made, although there are differing chromosomal levels present as in V. glabella Nutt. (n=6 or 12) (Clausen 1951) or as in V. sempervirens Greene (n=12 or 24) (Clausen 1951), to species pairs in which the polyploid differs only in quantitative characters from the diploid as in the pair V. stagnina (n=10) and V. elatior (n=20) (Clausen 1927), and finally to series like that in the Adnatae where V. selkirkii has n=12, V. japonica Langsd. and V. pinnata have n=24, and V. patrini has n=36 (Clausen 1929) and yet all are morphologically well-defined. It is clear therefore that polyploidy is important in the process of speciation within this genus.

Gershoy (1934) in his work on the relationship of chromosome number to morphology in Viola made several interesting observations.

He found that in general the species show a definite increase in nuclear and pollen diameter with increase in chromosome number, but that there is no uniform relation of chromosome number with organ size or other species characters. He attributed the sterility of tetraploids obtained from hybrid crosses to morphogenetic irregularities in the flower bud, rather than to problems of meiosis, for complete pairing of the chromosomes was observed in some specimens (Bold and Gershoy 1934). De Wet and Harlan (1972) state that complete bivalent formation is not as uncommon in autopolyploids as might be expected. Furthermore in an autotetraploid (V. elatior in the Rostratae) which maintains itself quite well in nature, Clausen (1927) commonly found tetravalents. It may be, therefore, that meiotic irregularities do not preclude or even bear much relevence to fertility in this group. Gershoy (1934) also found that in species with high chromosome numbers, which he therefore assumed to be polyploids, the chromosomes tended to be smaller than those in related diploids. Finally, it was his opinion that the variation introduced into members of the genus via hybridization and later backcrossings constrains the taxonomist to accept a fairly broad species concept.

An early example of an experimental approach to problems in plant evolution was provided by Ezra Brainerd (1904, 1906, 1924) in his work on hybridization in the genus Viola. This work began just after the turn of the century and has been continued to recent times by such workers as Gershoy (1928, 1934), Clausen (1930), Baker (1935), and Russell (1955). Amongst them they have proven that hybridization is a common phenomenon in this genus, and that

taxonomists often have been too quick to name as species specimens that are of hybrid origin. Crosses between species of different sections have been shown to be biologically unimportant, for in those cases in which an F_1 generation is formed, it is weak and sterile. Crosses between species of the same section, on the other hand, often produce F_1's and F_2's which are partly fertile. Thus, the groupings recognized on morphological grounds are corroborated by the genetic data. These findings are true of Viola adunca, for crosses are reported (Gershoy 1928) involving V. adunca and species of a relatively divergent ancestry (V. pedata, V. pinnata, V. rugulosa, V. canadensis) and the resulting hybrid seed did not germinate. Furthermore crosses within the Rosulantes (with V. rupestris and V. riviniana) resulted in vigorous hybrids which produced flowers but few seeds, all of which lacked embryos (Harvey 1966). Natural hybrids involving V. adunca are apparently uncommon, for Brainerd (1921) reports only one example, V. conspersa in Vermont being the other parent.

Chemosystematic methods have been introduced into the study of the genus by some workers in California who were seeking to identify the parents of an allotetraploid (Stebbins et al 1963). They followed procedures used by Alson and Turner (1959, 1962) in their pioneering studies, and were able to determine the species and subspecies which gave rise to the tetraploid. Their analysis consisted of paper chromatography of leaf extracts. The extracting medium and solvent systems were adopted in a modified form in part of the present study, for the extracted compounds are detectible by using either ultraviolet light or else a general phenol-detecting

reagent. They have the properties of good taxonomic characters and seem furthermore to resemble those used in Alston and Turner's work (1959) in Baptisia.

Paper chromatography is often effective in revealing the parentage of hybrids, for the chromatographic pattern of a hybrid is frequently a simple additive result of its parents' patterns (Alston and Turner 1962). Hidden variation between populations which may or may not parallel morphological variation has been described also, as in the chemical races of Baptisia (Alston and Turner 1963).

With regard to the identification of the spots developed, it was the opinion of Alston and Turner (1963) that even when unidentified, these compounds represent a significant pool of variation for taxonomic comparisons, but that when identified would contribute more precise data. Crawford (1970), using procedures very similar to those employed in the present study, isolated compounds which he identified as various flavonoids.

Clausen (1951) in summing up the experimental work on the genus states that the facts indicate "that its members have evolved large complexes of species that begin to take on the proportions and distinctness of separate genera. This is in part achieved through the building up of polyploid complexes with different basic numbers of chromosomes, for these complexes correspond to major morphological groups of the genus. In this manner, evolution is beginning to separate sections of the genus into new major evolutionary branches of the Viola tree (sic). Within most of these branches, evolution is still active in a reticulate pattern, but each branch is genetically so well separated from its neighbours that from now on it relies

wholly upon its own evolutionary resources."

Description and synonymy of Viola adunca.

Viola adunca occurs within the Rosulantes, as mentioned previously. Other species in this series are V. silvestris Lam., V. mirabilis, V. rupestris Schmidt, V. striata Ait., V. rostrata Pursh, V. riviniana, V. walteri House, V. reichenbachiana Jordan, and V. howellii Gray.

The original description of the species is as follows:

Viola adunca J.E. Smith

Rees Cyclopedia, 1817, volume 38, number 63 sub Viola.

"Stems simple, ascending. Leaves ovate, somewhat heart-shaped, obtuse, crenate, downy, dotted. Stipulas loosely fringed. Flower-stalks longer than the leaves. Nectary hooked. Brought by Mr. Menzies from the west coast of North America. This species has the size and habit of V. canina; and their stipulas, flower-stalks, and bracteas are similar. The calyx-leaves too are extended, in like manner, at the base. The whole of the herbage is minutely speckled, as in our last species (#62. V. maculata Cavan., from the Falkland Islands), as well as in canina. But the plant is more or less downy, and clearly distinguished by the strongly recurved form of the spur, which if straight would be as long as the lip. The two lateral petals are downy at the base. Perhaps this species is more akin to canina than to any other, and ought to stand near it; at least if the rubella (#61. V. rubella Cavan., native of Chili, South America) and maculata have no elongation at the base of their calyx."

A more complete description of the species is as follows:

Plants perenial, erect to spreading, caulescent, 4-5 cm long, often quite short at early flowering, with short to elongate slender rhizomes. Leaves long petioled, ovate to round-ovate, 2-5 cm long, 1-3 cm wide, cordate at base, crenulate, densely puberulent to nearly glabrous. Stipules linear-lanceolate, 3-10 mm long setulose, teeth mostly near base. Flowers slightly overtopping the leaves. Petal colour blue to deep violet, the lower three petals often with a whitish base, streaked with purple, the lateral pair white-bearded. Petals narrowly obovate 3-4 mm wide, curved upward or hooked, 4-7 mm long. Style head bearded with thick, short to fairly long hairs. Capsule 4-6 mm long. Seeds glabrous, dark brown, 1.5-2.0 mm long, 0.8-1.0 mm thick. Blossoms in May or early June in Alberta. Found in moist prairie, semi-wooded areas, and open ground near timber line throughout most of western North America, the prairies, and north-eastern North America. (Hitchcock et al 1961, Moss 1959). A distribution map is given in Fig. 3.

The species shows considerable variation over its extensive range, as might be expected, and several species have been erected on the basis of features which have come to be considered inadequate. Variations in pubescence, speckling of the leaves, and amount of curvature and relative length and shape of the spur have led to the publishing of names such as V. oxyceras (sharp-horned), V. odontophora (toothed), V. drepanophora (sickle-bearing), V. mammillata (nippled), and V. uncinulata (finely barbed) (Brainerd 1921).

Synonymy of Viola adunca J.E. Smith

V. labradorica Schrank., Denksch. Bot. Gesell. Regensb. 2:12. 1818.

V. punctata Schwein., Am. Journ. Sci. 5:67-68. 1822.

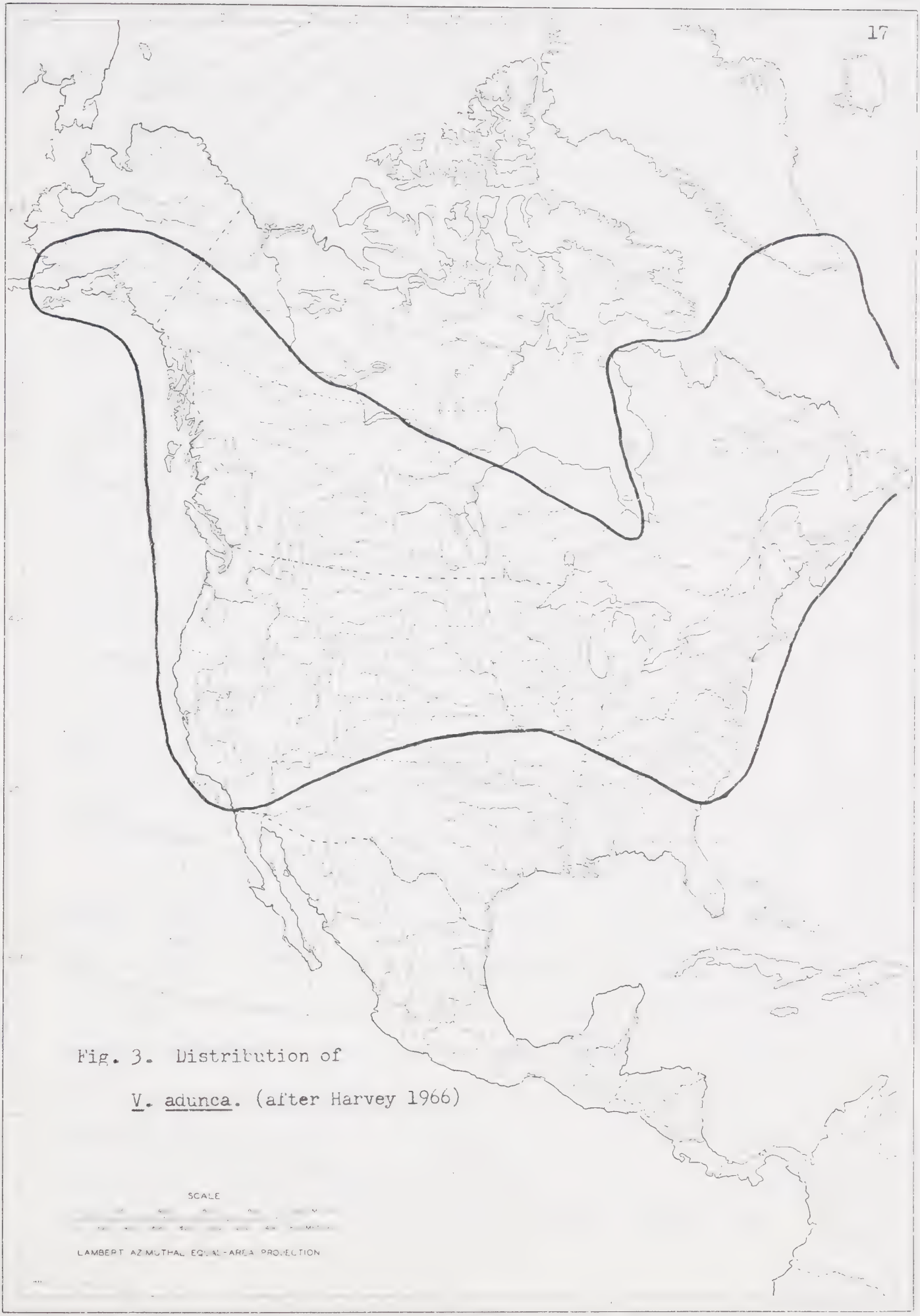


Fig. 3. Distribution of

V. adunca. (after Harvey 1966)

V. muhlenbergiana Hook., Fl. For. Am. 1:178. 1830.

V. longipes Nutt. in T. and G., Fl. N. Am. 1:140. 1838.

V. canina var. adunca Wats., Bot. Calif. 1:55. 1880.

V. canina var. longipes Wats., Bot. Calif. 1:56. 1880.

V. canina var. oxyceras Wats., Bot Calif. 1:56. 1880.

V. oxyceras Greene, Pittonia 3:255. 1897.

V. subvestita Greene, Erythea 5:39. 1897.

V. montanensis Rydb., Mem. N.Y. Bot. Gard. 1:263. 1900.

V. odontophora Rydb., Mem. N.Y. Bot. Gard. 1:264. 1900.

V. monticola Rydb., Mem. N.Y. Bot. Gard. 1:264. 1900.

V. filipes Greene, Pittonia r:289. 1901.

V. retroscabra Greene, Pittonia 4:290. 1901.

V. bellidifolia Greene, Pittonia 4:292. 1901.

V. verbascula Greene, Leaflets Bot. Obs. 2:32. 1910.

V. drepanophora Greene, Leaflets Bot. Obs. 2:32. 1910.

V. mamillata Greene, Leaflets Bot. Obs. 2:33. 1910.

V. oxysepala Greene, Leaflets Bot. Obs. 2:34. 1910.

V. uncinulata Greene, Leaflets Bot. Obs. 2:97. 1910.

Clausen (1929) believes that Viola adunca should be placed under V. rupestris Schmidt of Eurasia but admits that some V. adunca specimens are more than a little different from V. rupestris, and that these are different in spur and style features. Despite the significance generally attached to the style characters, he favours the view that V. adunca and V. rupestris are conspecific. Brainerd (1913) in considering the same two groups comes to the opposite conclusion, and presents a table of seven points of difference involving characters of the leaves, stipules flowers, bracts, petals, spurs

and capsules. Harvey (1966) reports a high percentage of bivalent formation in the meioses of hybrids of V. adunca and V. rupestris, but he also states that the hybrids are completely sterile and that because of their additional morphological and geographical differences, the two species should be retained. The work of Brainerd and Harvey appears conclusive, and V. adunca is generally accepted (Fernald 1950, Hitchcock et al 1961, Hulten 1968) as specifically distinct from its Eurasian ally.

Viola adunca was for some time confused with V. canina, and recognized as a variety of that taxon. However, the facts that there is a rosette in V. adunca, and that it is a North American species serve to separate it from V. canina (Becker 1923).

Cytology and phytogeography of Viola adunca.

The chromosome counts of Viola adunca made by previous workers are recorded in Table IV. The presence of a tetraploid race and its possible geographical separation from the diploid did much to suggest the present study.

Table IV. Chromosome counts of Viola adunca.

Locality	2n	Authority
Burlington, Vt.	20	Gershoy 1934, Harvey 1966
Mt Jacques Cartier, Que.	20	Harvey 1966
Mather, Cal.	20	Harvey 1966
San Bruno Mts, Cal.	20	Clausen 1929
Mt. Vision, Cal.	20	Clausen 1929
Oregon	20	Clausen 1964
Vancouver, B.C.	20	Clausen 1964
Colorado	20	Clausen 1964, Packer (unpublished)
Wyoming	20	Packer (unpublished)
Edmonton, Alta.	40	Packer 1964
Sidney, Man.	40	Taylor and Brockman 1966

Studies in the phytogeography of the genus have yielded data bearing on evolution within the genus, as indicated above, and some data gathered during the present study may give an insight into another problem in this field. Preliminary results from the study of the distribution of the two chromosome races indicated that there might be within Viola adunca an example of the replacement of diploid by polyploid populations in recently disturbed (in this case, glaciated) areas.

The interpretation given the observation of the differing geographical distributions of polyploid races of a variety of species has changed several times since the phenomenon was first noted. Hagerup (1931, 1933) proposed that environmental severity and

polyploidy are causally related. Tischler (1935), and Love and Love (1943) collected evidence which suggested to them that the incidence of polyploidy in a flora rises with increasing latitude and they postulated that polyploids are hardier than their diploid ancestors. The work of Strelkova (1938) in Alopecurus L, and Hagerup (1933) in Vaccinium uliginosum L. showed that chromosomal races often differ ecologically and geographically. At the same time as this work was being done, other workers (Clausen, Keck and Hiesey 1945, and Bowden 1940) were demonstrating by transplanting experiments that the greater tolerance of polyploids is questionable. A consideration of these and other ideas is found in Johnson, Packer and Reese (1965). As Stebbins (1971) points out, the data that had been collected demonstrated correlations between frequency of polyploidy and latitude, coldness, glacial effects, and isolation, but the question of which correlation was significant remained open. Work carried out by Johnson and Packer (1965) in artic North America supports the idea that the percentage of polyploidy is related to the effects of glaciation, and more generally, to the amount of current or recent disturbance of climate and soil. This work, done at Ogotoruk Creek, Alaska, indicated that in habitats which have undergone many changes in climate and soil characteristics, polyploids are favoured by natural selection. Randhawa and Beamish (1972), working with Saxifraga ferruginea Graham in British Columbia and adjacent areas, have found that diploid populations occur in southern unglaciated regions and suspected refugia, but that areas known to have been glaciated (mainland B.C.) yield only polyploid specimens.

Stebbins (1950) suggests that critical studies bearing on

this phytogeographical phenomenon should take the form of an analysis of the distributions of groups which are known to contain both polyploids and their parental groups, and that the comparisons of whole floras done in the past can be misleading. The present study has the recommended form, and may offer some data free of the complications found in a study of plants with contrasting and varied growth forms and life cycles.

The last author, in the same work, states as his opinion that "polyploids ... from crossings between races or subspecies of a species are likely to possess wide ranges of tolerance of climatic and edaphic conditions, as has been demonstrated experimentally for many interspecific and interecotypic hybrids. They are thus ideally suited to the colonization of areas newly opened to plants ...." In a later paper (1971) he speculates on the origin of polyploids invading glaciated North America. He suggests that at the ice margin during both its advance (which began some 40,000 years ago in the case of the Wisconsin glaciation) and its retreat, the more severe climate would cause a selection of hardier members of a species, and perhaps cause the evolution of poplations closely adapted to only a few subdivisions of the former habitat of the species. About 20,000 years ago, the retreat of the ice began, and the decay extended over the next 10,000 years. This retreat was irregular (Flint 1971), so that ecotypic extremes could have occurred close to one another. Hybrids between these extremes, and polyploids of the hybrids would have produced a rich and variable gene pool, and from this would be chosen the genotype best adapted to the habitats emerging as the ice retreated.

MATERIALS AND METHODS

Specimens

Specimens from the following herbaria were studied: The Gray Herbarium of Harvard University (GH), the Herbarium of the University of California at Berkeley (UC), the Dudley Herbarium of the University of California at Stanford (DS), the Herbarium of the University of British Columbia (UBC), the Herbarium of the New York Botanical Gardens (NY), the Herbarium of the University of Alaska (ALA), the Herbarium of the University of Colorado (COLO), and the Herbarium of the University of Alberta (ALTA). (Abbreviations as given in Index Herbariorum 1964).

Collecting techniques

Living plant material was collected in the field and brought back to the greenhouse at the University of Alberta during the 1970 and 1971 field seasons. Almost all of Alberta was sampled and one field trip was made to each of the adjacent provinces and states with a view to collecting over as wide an area as possible. In addition, live material was provided by Mr. J. Traquair from the Ontario Peninsula.

Collections were usually made within a few yards of the road being travelled, but in most cases beyond the obvious influence of the road. A grid pattern was not followed, although it would have provided the most complete data on distribution. The size of the

study area made such a survey impractical. At all collecting sites an attempt was made to collect three specimens as widely separated as possible to avoid the collecting of specimens derived from a single plant. The specimens were dug up with their roots intact and transferred either to plastic pots directly or to bags and subsequently to pots in Edmonton. By the end of the summer of 1971, over 300 living specimens from about 150 sites had been collected. In addition, specimens for pressing were taken at these and other sites, and are now filed in the Herbarium of the University of Alberta.

Cultivation

Plants in the greenhouse are maintained under the following conditions. The temperature is kept close to 18° C, natural lighting conditions prevail in summer, and in winter artificial lights keep the daylength at about 15 hours. The humidity is kept at 50% or higher. The specimens did well under these conditions, and less than 15 plants, most of them recently transplanted, were lost in the greenhouse. The roots of this species are not markedly spreading, and since the soil in which it grows is usually cohesive, it can be dug up in a 6 inch diameter soil block, and will suffer little damage.

It is interesting to note that in the spring of both 1971 and 1972 the diploid specimens flowered about two weeks before the tetraploids, although the two were maintained under identical conditions. Thus this phenological difference, which doubtlessly arose as an adaptation to the later springs that occur at higher latitudes, is genetically determined.

Guard cell and pollen measurements

Measurements of guard cell lengths were made on epidermal peels of lower leaf surfaces. Dried leaves were soaked for two or more hours in water to which a small amount of detergent had been added. The peels were mounted in water and measured using a graduated eyepiece fitted to a microscope. Initially some 30-35 pairs of cells were measured per specimen, but it eventually became obvious that in most cases 10-15 measurements were sufficient. Over fifty cytologically known specimens were examined.

Measurements were also made of some 150 pollen grains from fresh flowers of each chromosome race.

Cytology

Root tips were taken from the plants after they had established themselves in their pots. These apices were treated with a 0.002 molar solution of 8-hydroxy-quinoline (0.116 grams in 400 ml of water) for three to four hours at 14° C ($\pm 2^{\circ}$ C) (Tjio & Levan 1950). This solution halts mitosis at metaphase, leaving the shortened and thickened chromosomes attached to the metaphase plate. The root tips were then washed in distilled water for five minutes, and then transferred to a staining solution of 9 parts acetic orcein to 1 part 1N HCl. This solution was warmed over a bunsen burner 3-5 times during a 30 minute period, and the root tips were then placed in a drop of 45% acetic acid on a slide and squashed between it and a cover slip. This procedure spreads the cells apart and usually causes disruption of the metaphase plate, leading to the dispersal of its chromosomes throughout each cell. A mixture of Canada

balsaam and paraffin was then melted and applied about the edges of the cover slip, thereby rendering the slide relatively permanent. The chromosome numbers were then counted using a Vickers or Olympus microscope with a green filter in place.

Pollen mother cells were studied to obtain information on the regularity of meiosis in the tetraploid. The anthers of young, unopened chasmogamous flowers were dissected open in a drop of acetic orcein, warmed 3 or 4 times during an hour staining period, and then squashed under a cover slip and examined under the microscope. Meiosis occurs in this species when the flower buds are from 1 to 2 mm. long. The slides have been placed on file in the Herbarium of the University of Alberta.

Bagging

The possibility that apomixis might occur in the tetraploid members of the species was examined by means of a bagging experiment. From 15 specimens a few young chasmogamous flowers in which the anthers had not yet dehisced were emasculated and covered in two or three layers of gauze netting to prevent cross-pollination. After two weeks the netting was removed and the size of the ovary examined.

Chromatographic techniques

Five or six leaves were ground with acid-cleaned sea sand, using mortar and pestle, and the resulting powder was placed in a small vial to which was added 0.5 ml. of extracting medium (see below); the solution was spotted on to half-sheets of Whatman Number 1 chromatographic paper. Approximately 0.2 ml/sheet was applied using a capillary tube. Each application was allowed to dry

before more material was added, and the spotting was continued for about 45 minutes, or less if a dryer was used to blow cool air over the sheets. The sheets were then placed in a chromatography tank, and one of the A solvents (see Table V) was allowed to descend the paper. The sheets were then removed, allowed to dry, trimmed, and replaced in the chromatography tank with the corresponding B solvent. At the completion of this run the sheets were again removed, allowed to dry and then examined under longwave (3660A) ultraviolet light. Fluorescent spots were circled, their colours noted, their R_f values (ratio of the distance travelled by the spot to that travelled by the solvent front) calculated, and their intensity estimated on a scale of 1 to 4 based upon the intensity of the chlorophyll spot (4). In an effort to characterize the spots, the chromatographs were subjected to a variety of chemical tests (taken from Smith 1969). The facts that phenolic compounds are extractable by the method used, and that they sometimes fluoresce blue, green, purple or yellow under ultraviolet light indicated that the compounds on the chromatographs might be phenolics. A ferric chlorideferricyanide test was therefore conducted. A 0.3% solution of $K_3Fe(CN)_6$ was mixed with a 0.3% solution of $FeCl_3$ in water and the chromatographs were dipped through the solution, and then washed with dilute HCl and water. On drying, phenolics appear as blue spots. The test is considered sensitive for it detects amounts of less than 1 ug. p-Nitrobenzenediazonium tetrafluoroborate (Eastman Kodak) was also used to test for the presence of phenolics. The appearance of brown spots marks a positive test.

Treated and untreated chromatographs were exposed to NH_3

fumes to determine if any colour changes occur.

A 1% solution of iodine in carbon tetrachloride was used to test for alkaloids primarily, although some other classes of organic chemicals for example the imidazoles, are also stained by this solution. The chromatographs were dipped in the solution and allowed to dry in a fume cabinet. Alkaloids are stained yellow and brown.

As a test for lipids, chromatograms were placed in a dry tank containing a few crystals of iodine. The iodine vapour stains lipids (and some unsaturated compounds as well) varying intensities of yellow.

No tests were carried out for indoles, purine and pyrimidine derivatives, or steroids for all of these compounds absorb ultraviolet light and appear as dark spots. No dark spots occur in these chromatographs (see Table VII).

Solvent system I (Table V) involved extraction with absolute ethanol for 20 minutes, and then development by descending chromatography first in 15% acetic acid (v/v) and then in a mixture of n-butanol 63:acetic acid 10:water 27. This solvent system proved unsatisfactory because the first solvent ran so fast that the extract was spread into a long streak in which individual spots were not recognizable, and the resolution was not improved by the run in the second direction.

Solvent system II was unsatisfactory for the same reasons.

Other workers (Stebbins et al. 1963) in the genus had used a 1% solution of HCl in methanol in their extractions, and the solvent system labelled III in Table V. Better separation was obtained for

Table V. Solvent systems used in chromatography

Extracting medium	Solvent A	Solvent B
I. Abs. Ethanol (20 min)	15% v/v HAc	n-butanol 63: HAc 10: water 27
II. Abs. Ethanol (20 min)	15% v/v HAc	iso-propanol 160ml: formic acid 8 ml: water 40ml
III. 1% HCl in methanol (12 hours)	t-butanol 3: HAc 1: water 1	HAc 15: water 85
IV. 1% HCl in methanol (12 hours)	t-butanol 3: HAc 1: water 1	NaAc 10 g: water 400 ml: HAc 1 ml

the first direction, for as Smith (1969) states, this solvent allows no diffusion or elongation of the spots during the chromatography, and is valuable as the first solvent in 2-way chromatography. However the acetic acid run was again somewhat fast so that streaking occurred.

A modification of this last technique proved acceptable (solvent system IV). By the addition of sodium acetate (at a concentration of about 5% w/v), the speed of the second run was reduced and adequate separation of the compounds was obtained. The time needed for the first directional run was about 15 hours, and for the second, 1.5-2.0 hours. The result is a chromatograph approximately 9" x 7" which shows about 20 fluorescent spots under ultraviolet light.

Some doubt existed as to whether the compounds being isolated using the HCl-methanol extract were the same as those obtained in absolute ethanol, or rather were degradation products of various sorts formed because of the acid in the extracting solution. The

technique was therefore tested in the following way. From one extract solution, chromatographs were run at times of 1 hour, 4 hours, 8 hours, and 24 hours after extraction. In addition from the same dry material an extract was made using absolute ethanol, and after twenty minutes the extract was spotted onto chromatographic paper. The results of the comparison of the two methods will be discussed below.

Thin layer chromatography (TLC) was investigated as a possibly valuable mode of inquiry, because of the relative speed of the technique in comparison to paper chromatographic methods. Cellulose powder (Cellulosepulver MN 300, distributed by Brinkmann Instruments) was mixed with water (15 g. cellulose with 90 ml. distilled water for 4 plates) in a blender and poured into a Brinkmann spreader set for an aperture of 0.5 mm. The spreader was then drawn quickly across ethanol-cleaned glass plates to give an even film of cellulose.

Silica gel was also tested as a possible medium. 25 g. of Silica Gel (distributed by Camag) were mixed with 60 ml. distilled water and applied to the 4 plates as above.

The spotting was done using a capillary tube and the solvents described previously were poured into a covered glass tank and allowed to rise up the plates. The resulting TLC plates were examined under ultraviolet light and a tracing was then made of each spot pattern.

TAXONOMY

Morphology

The diploid race is widespread across North America, as previously indicated, and as the list of synonyms suggests, it is variable in its morphology. The search for characters separating the two races on morphological grounds was not aided by the variability in the parent group. Thus flower size, petal and leaf shapes and dimensions, and growth habit vary considerably in this taxon, but have been found to vary independently of the chromosome number. Characters intermediate between Viola adunca and other Viola species have not been found. However, after specimens had been grouped according to chromosome number, some character states were found to be diagnostic.

A limited biometric approach was made during the investigation of variation. Measurements were made of the variation of leaf tooth number versus length of the leaf, of stipule length, and of length of sepals, and the results of the latter two studies are presented graphically in Figures 4 and 5. In each case 100 measurements of the character were made. There is no discernible difference between the two races on these bases, although variation in characters of this nature is not uncommon between closely related taxa. A two-sample t-test shows that in each case the means of the two populations are not significantly different at the 5% level.

There appears to be a good correlation of chromosome number

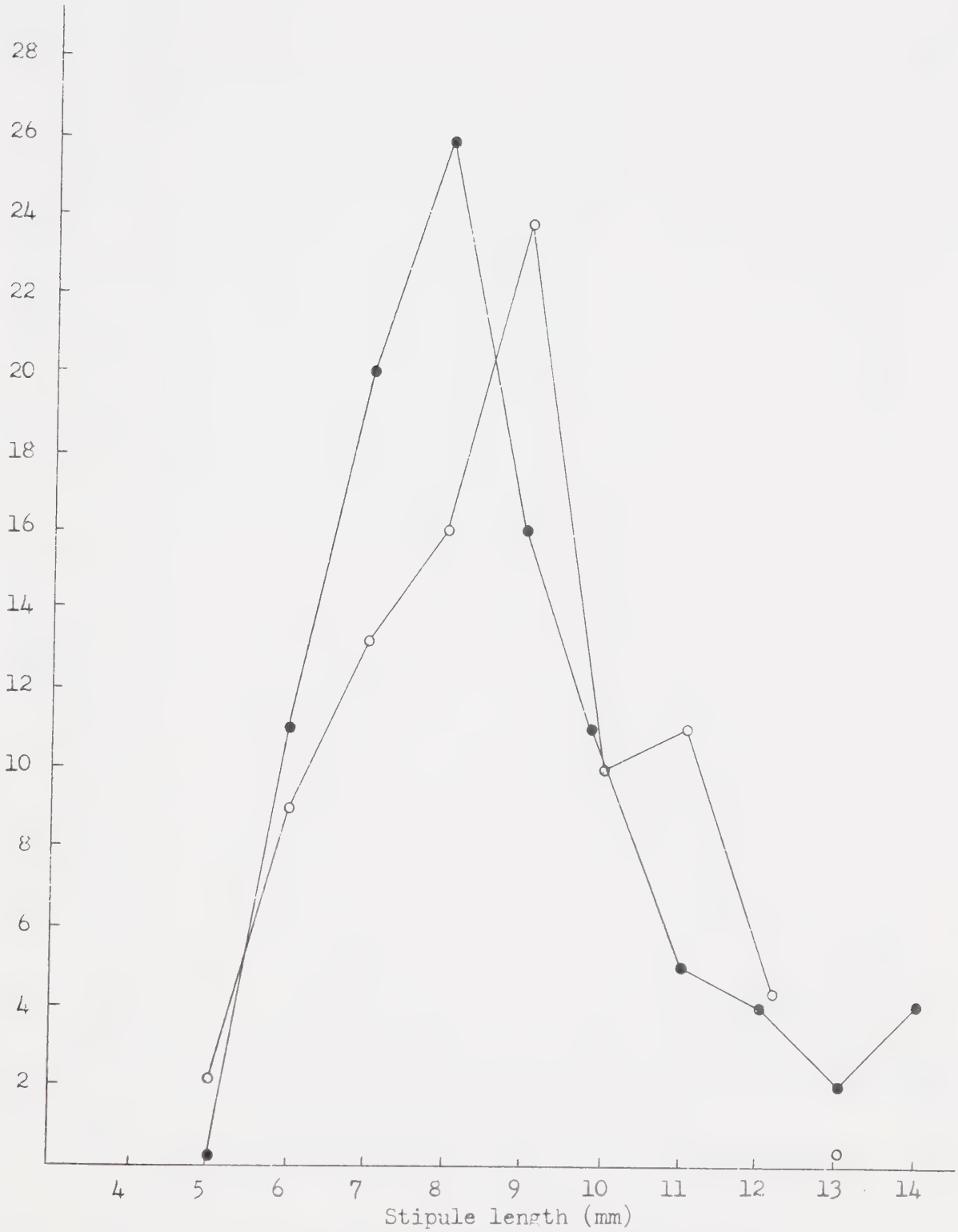


Fig. 4. Stipule length frequencies in Viola adunca.

o - diploid

● - tetraploid

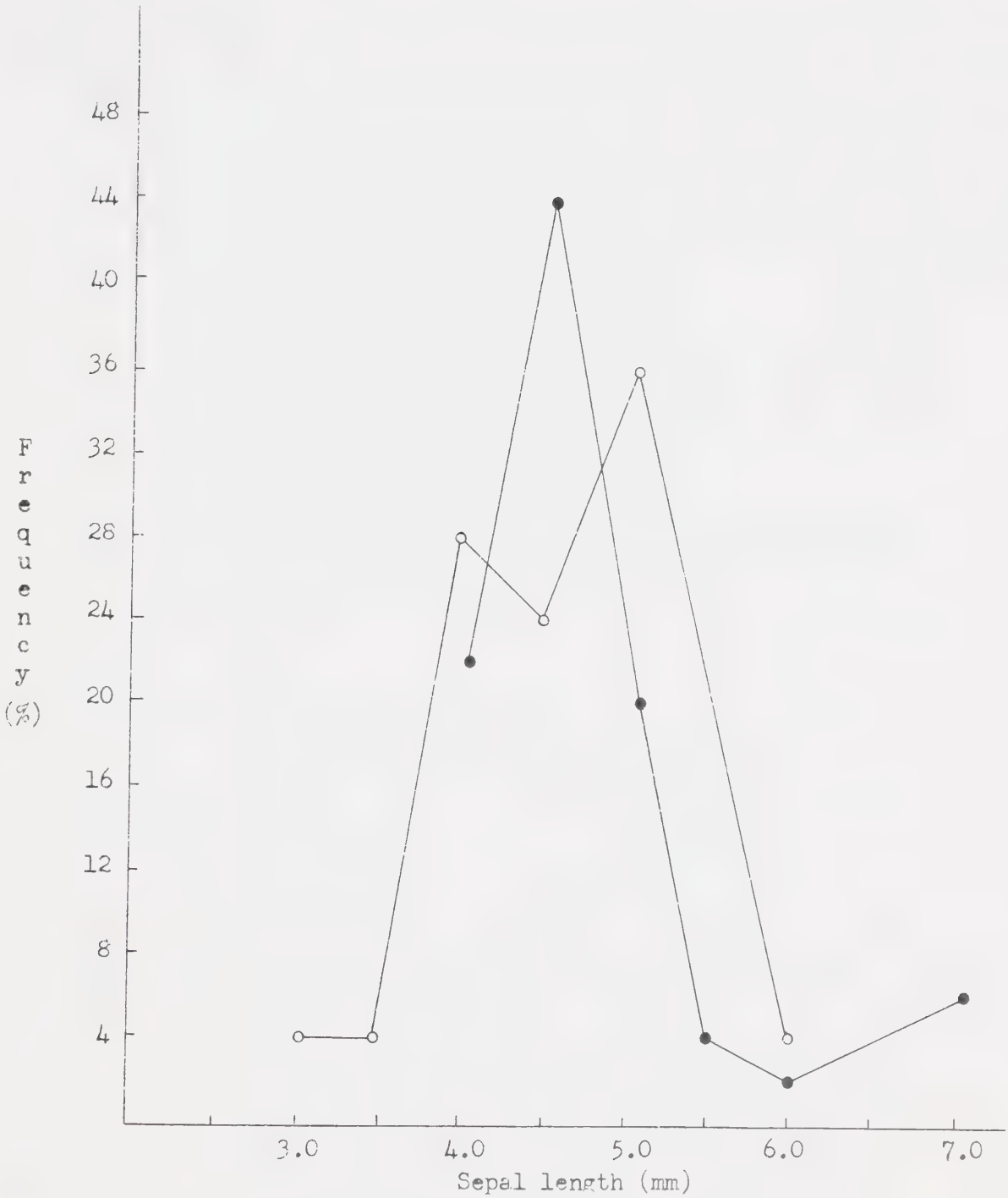


Fig. 5. Sepal length frequencies in Viola adunca.

o - diploid

● - tetraploid

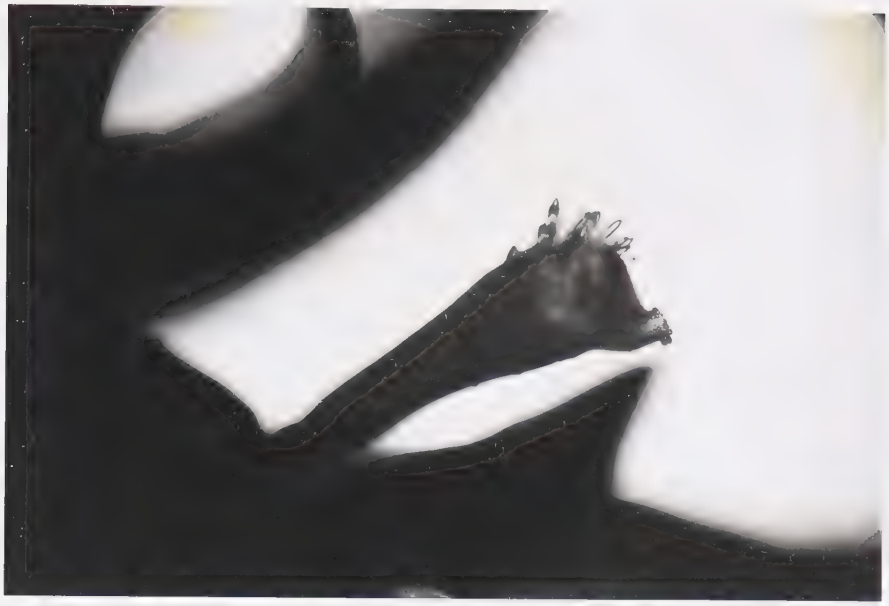
with morphology of the style head. The tip of the style bears, in both chromosomal levels, projections of which the shape and length are different in the two groups (Plate II and Fig 6). The hairs function as part of the insect-guiding mechanism (Becker 1935) and, together with the beards on the lateral petals, direct pollinators past the stigma and stamens down into the spur, in which nectar is found. In the diploid, the projections are roughly cylindrical and as much as one-sixth the width of the style, while in the tetraploid they are either short-conical or globular, and about one-tenth or less the width of the style head. Often too, the diploids have a more crowded arrangement of the projections.

Both diploid and tetraploid exhibit variation from an almost smooth style head through to the typical condition for their chromosomal level. Thus, a diploid specimen with poorly developed projections can be mistaken for a tetraploid. However, an examination of other flowers on the same plant may clarify the specimen's identity. In pressed specimens, the projecting hairs are often so flattened that their size and shape is difficult to determine, and soaking in water and detergent may be necessary.

Sometimes the pubescence of the leaves will give an indication of the polyploidal level of a specimen. Both races vary from glabrous to quite pubescent, as do all the other species in the genus in North America (Brainerd 1913), but in the diploid the longest hairs found are up to 0.30 mm, while in the tetraploid, hairs longer than 0.22 mm have not been observed. In many cases, nevertheless, it is impossible to determine the chromosome number from pubescence because the specimen is glabrous or bears hairs shorter

Plate II. Style apices in V. adunca. (X25)

Diploid



Tetraploid

Diploid.

Tetraploid.

Fig. 6. Ranges of variation in protuberances of stylar apices in the diploid and tetraploid races of Viola adunca.

than 0.22 mm.

Guard cells

The guard cells in the two races were found to differ in size, the diploids being the smaller of the two. A two-sample t-test shows that at the 1% level, the two means are significantly different (s=1.82, t=12.1). This type of distinction is common in polyploid series (Davis and Heywood 1963) and has been attributed to an increased size of the nucleus. In the majority of cases, only a relatively small number of cells need be measured, for the overlap in length involves no more than about twenty-five percent of the guard cells measured (Fig. 7).

Pollen size

In many genera it has been recorded that pollen size varies with chromosomal level, and Gershoy (1934) found this to be the case in his studies in *Viola*. Measurements of the intact triangular pollen grains of *V. adunca* are consistent with these findings (Fig. 8). A two-sample t-test shows that at the 1% level the means of the two populations are significantly different (s=1.82, t=12.1). Thus the chromosomal level of a specimen may be determinable from the size of its pollen grains.

It would seem most likely, in view of the essential identity in morphology of the two races that the tetraploid has arisen from the diploid by autopolyploidy. This conclusion is supported by Gershoy's work (1934), for he found that crosses wider than intra-subsectional produced sterile hybrids, and he states that as a rule he has found that doubling of the chromosome number does not confer

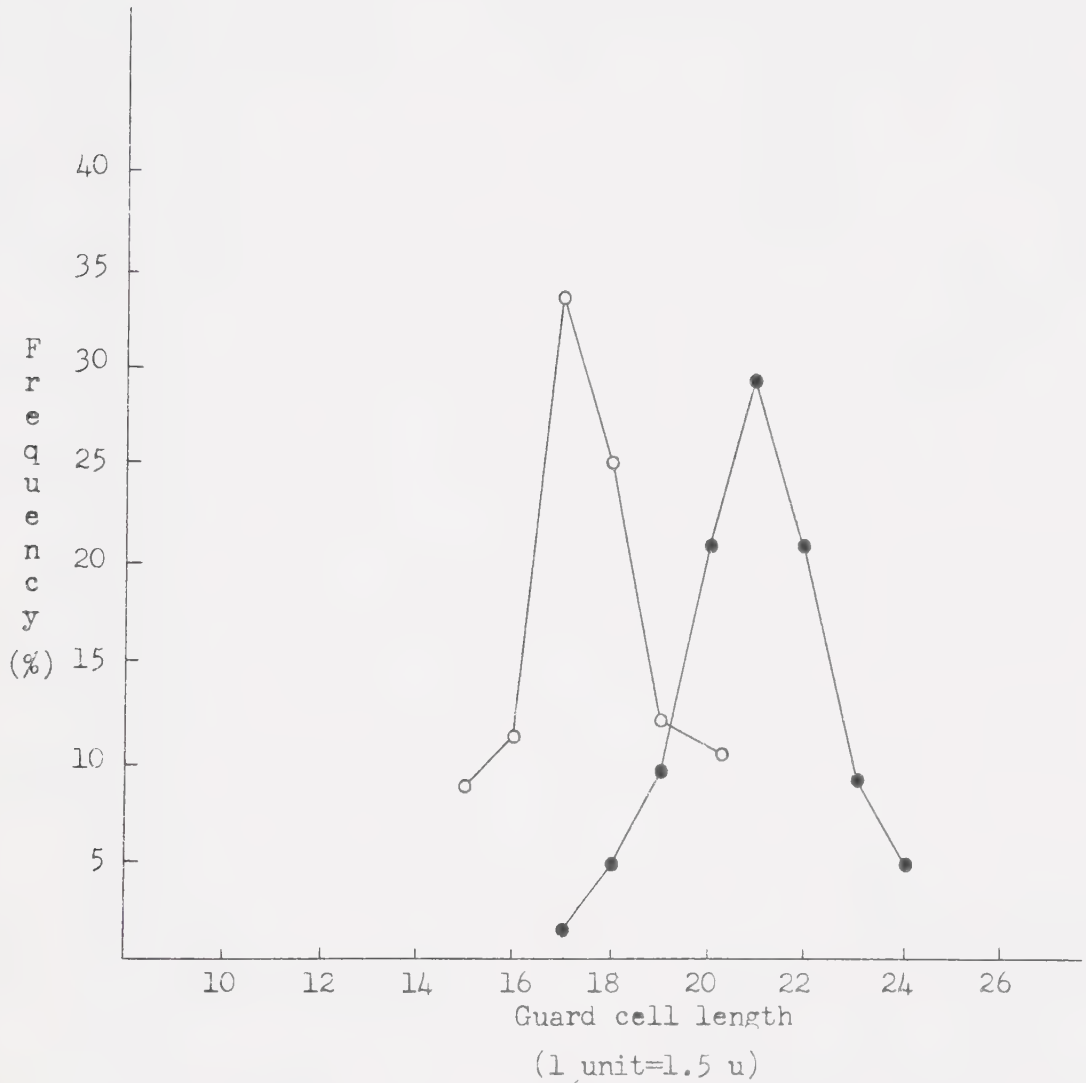


Fig. 7. Leaf guard cell length frequencies in Viola adunca.

o - diploid

● - tetraploid

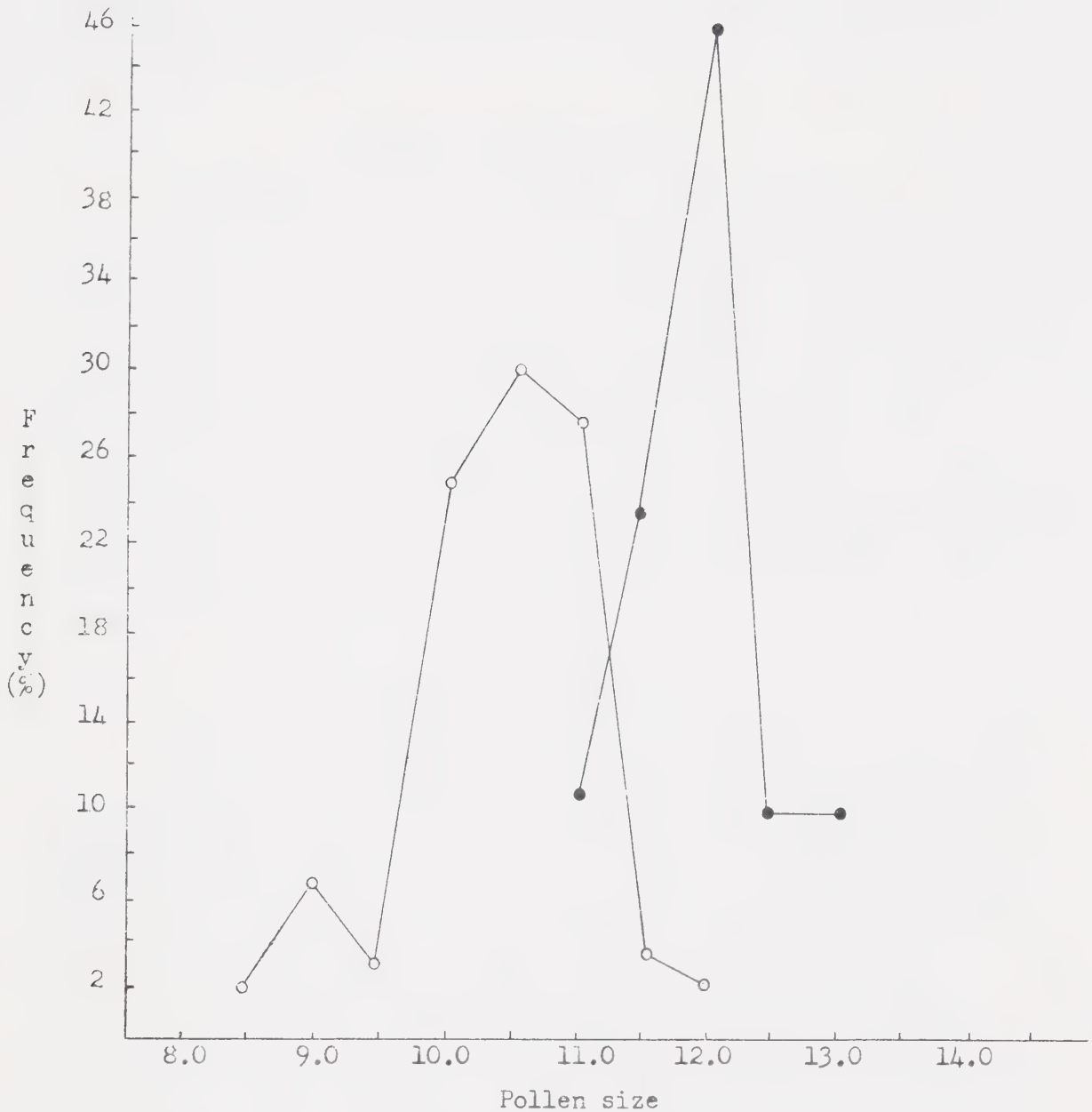


Fig. 8. Pollen length in the two chromosome races of Viola adunca. (1 unit=0.38 u)

o - diploid

● - tetraploid

fertility upon hybrids. Since Viola adunca is the only member of its subsection found in north-central North America, there seems little likelihood that the tetraploid arose from a crossing wider than intervarietal.

Cytology--somatic counts

The chromosomes of the diploid and tetraploid races (Fig. 9) were found to differ slightly in size when treated in the same way. After a three hour treatment at 14° C the 8-hydroxy-quinoline causes the contraction of a diploid specimen's chromosomes to 2.8-3.0 microns and a tetraploid specimen's to 2.5-2.6 microns. All the chromosomes are metacentric. Three chromosomal levels were observed (2n, 3n, and 4n); no aneuploidy was in evidence. A list of chromosome counts is presented in Table VI.

Cytology--pollen mother cells

Meiosis in the diploids has been observed to be conventional, as was expected. In the tetraploid race, because of the apparently autopolyploidal nature of its origin, there was some expectation that quadrivalents would be observed. However, meiosis was regular (Fig. 10)--only bivalents were observed. Thus, it would appear, some diploidization has occurred, rendering homologous chromosomes incompatible. This process was first reported by Riley (1962) in Triticum, but the steps involved in Viola adunca are completely unknown. The regularity of meiosis may also be interpreted as evidence for the origin of the tetraploid race from a crossing between different varieties of the diploid. If the two populations had been evolving separately for some time, then their chromosomes might have

Fig. 9. Somatic chromosomes in Viola adunca determined from root tip meristems.
a. 2n=20
b. 2n=40 (X 1000)

Fig. 10. Meiotic chromosomes in Viola adunca determined from pollen mother cells. (X 1300)
a. metaphase I in the diploid
b. metaphase I in the tetraploid

undergone changes, such as translocations or inversions, which would discourage quadrivalent formation at meiosis.

Table VI. List of specimens used in cytological study.

Diploid Specimens.

Alberta: Coleman, GDM 386, June 19/71; Woolford Prov Park, GDM 359 June 3/71; Waterton National Park, GDM 568, 569 Sept 15/71; Sofa Mt, GDM 570 Sept 15/71; Reesor Lake, GDM 355, 356 June 1/71; Cypress Hills, GDM 357 June 1/71; N of Brooks, GDM 478 July 27/71; W of Coleman GDM 364 June 3/71; Three Hills, GDM 461 July 26/71; Gooseberry Lake Prov Park, GDM 541, 542 Aug 15/71; Saskatchewan: Mankota, GDM 502, 516 July 28/71; W of Kildeer, GDM 506, 510, 511, 512, 513, 514, 515 July 28/71; Woodrow, GDM 507 July 27/71; S of Moose Jaw, GDM 508 July 28/71; British Columbia: Fernie, GDM 365 June 4/71; Cranbrook Prov Park, GDM 366 June 5/71; E of Yahk, GDM 367 June 5/71; W of Creston, GDM 368 June 5/71; W of Nancy Green Lake, GDM 369 June 5/71; W of Greenwood, GDM 370 June 6/71; Bridesville, GDM 371 June 6/71; W of Princeton, GDM 372 June 6/71; Skookumchuk, GDM 441 July 11/71. Ontario: Hensall, GDM 571, 572, 573, 574, 575, 576, 577 Oct 10/71. Montana: S of Aden, GDM 358 June 2/71; S of Carway, GDM 395 June 2/71; Glacier Park Entrance, GDM 394, 413 July 9/71; Glacier Park Boundary, GDM 425, 436 July 10/71; Coram, GDM 426 July 10/71; Olney, GDM 427 July 10/71; Eureka, GDM 437 July 10/71.

Triploid specimens.

Alberta: W of Cardston, GDM 360 June 3/71; S of Knight Lake, GDM 361 June 3/71; Bauerman Creek Road, GDM 362, 363 June 3/71; Robb, GDM 550 Aug 17/71; Forestburg, GDM 539 Aug 14/71; Cypress Hills,

Table VI. List of specimens used in cytological study.

Triploid specimens. (continued)

GDM 357 June 2/71; Reesor Lake, GDM 352 June 1/71. Saskatchewan: Borden, GDM 535 June 29/71.

Tetraploid specimens.

Alberta: Rock Lake, GDM 268 Aug 9/70; S of Rock Lake, GDM 270 Aug 9/70; Gainford campsite, GDM 271, 272 Aug 12/70; Red Deer campsite, GDM 348 May 31/71; Nojack, GDM 273, 274, 275 May 31/71, and 61 June 9/70; Carrot Creek, GDM 276, 277, 278, 279 May 31/71; Marlboro, GDM 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291 Aug 13/71; Luscar, GDM 66, 67 June 9/70; Pembina River campsite, GDM 68 June 10/70; Brazeau River campsite, GDM 69 June 10/70; Elk River, GDM 71 June 10/70; Brown Creek, GDM 72 June 10/70; Jasper Airfield, GDM 74 June 10/71; Robb, GDM 550 Aug 17/71 and GDM 75, 76, 77 June 11/70; Mt. Edith Cavell, GDM 292, 293, 294, 295, 296, 297 Aug 12/70; Jasper Park Entrance, GDM 298, 299 Aug 13/70; S of Mt Edith Cavell, GDM 453 July 12/71; Bow River, GDM 381 June 18/71; S of Bow River, GDM 382, 383 June 19/71; Livingstone Falls, GDM 384, 385 June 19/71; Waiparous Creek, GDM 387, 388 June 20/71; Brazeau River, (Traquair) GDM 549 Aug 19/71; James River campsite, GDM 389, 390 June 20/71 and (Traquair) GDM 544, 545 Aug 19/71; Lambert Creek, GDM 546 Aug 19/71; Elk Creek, GDM 391 Aug 19/71; Coalspur campsite, GDM 548 Aug 17/71; Ram River Falls, GDM 392 Aug 17/71 and (Traquair) GDM 547 Aug 19/71; Banff, GDM 451 July 12/71; Columbia Ice Fields, GDM 452 July 12/71; Cypress Hills, GDM 319, 320, 321 May 9/71; Reesor Lake, GDM 352 June 1/71; S of Cross Lake, GDM 300 Aug 18/70; S of Slave Lake, GDM 302, 303, 304,

Table VI. List of specimens used in cytological study.

Tetraploid specimens. (continued)

305 Aug 18/70; Fort Assiniboia, GDM 306, 307 Aug 18/70; Whitecourt, GDM 551 Aug 26/71; Grande Prairie, GDM 552 Aug 27/71; Hythe, GDM 553 Aug 27/71; Boundary Lake, GDM 558 Aug 30/71; Clear Prairie, GDM 559 Aug 30/71; Eureka River, GDM 560 Aug 30/71; Whitelaw, GDM 561 Aug 30/71; Manning, GDM 562 Aug 30/71; Hotchkiss, GDM 563, 564 Aug 31/71; Keg River, GDM 565, 566 Aug 31/71; Peace River, GDM 567 Aug 31/71; W of Edmonton, Packer Acc no 12736 April 1959; New Norway, GDM 460 July 26/71; Bittern Lake, GDM 459, 467, 468, 469 July 26/71; Vermilion, (Hanson) GDM 455 July 14/71; Lac La Biche, (Scott) GDM 378, 379 June 6/71; Cold Lake, (Scott) GDM 380 July 7/71; Viking, GDM 357, 358 Aug 14/71; Big Knife Prov Park, GDM 540 Aug 8/71; Innisfree, GDM 543 Aug 15/71. Saskatchewan: Au'Appel, GDM 531, 532 July 29/71; Touchwood Hills, GDM 533 July 29/71; Lanigan, GDM 534 July 29/71; Cypress Hills, GDM 479, 480, 501 July 27/71. British Columbia: N of Kamloops, GDM 373 June 8/71; Little Fort, GDM 374 June 8/71; N of Clearwater, GDM 375 June 8/71; S of Avola, GDM 376 June 9/71; S of Tete Jaune, GDM 377 June 9/71; Roosville, GDM 438 July 11/71; Jaffray, GDM 439, 440 July 11/71; Canal Flats, GDM 442, 443 July 11/71; Radium Hot Springs, GDM 444 July 11/71; S of Dawson Creek, GDM 554 Aug 27/71; N of Wonowon, GDM 555 Aug 27/71; Fort St. John, GDM 556, 557 Aug 29/71.

Voucher specimens of each of these counts have been deposited in the herbarium of the University of Alberta (ALTA). Collection made by the author are indicated by "GDM."

Bagging

The young tetraploid flowers that were tested for apomixis by being emasculated and bagged did not produce seeds. Thus the tetraploid does not produce seeds without fertilization. Some difficulty with fungal growth was encountered, but over 20 flowers on the 15 plants survived and did not develop swollen ovaries. The frequency of sterility in autotetraploids is regarded in the literature (Stebbins 1950) as favouring the formation or reinforcement of methods of vegetative reproduction. However, the reductive step from self-pollinating, cleistogamous flowers to apomictic forms does not seem to have taken place.

Paper chromatography

The results of the experiment comparing the extracting properties of ethanol versus methanol-HCl proved the superiority of the methanol-HCl combination. All the spots developing from the ethanol preparation were present in all the results of the methanol-HCl preparation. Furthermore, it was also possible to determine the spot colours of the methanol-HCl tests, whereas in the ethanol tests, the spots were somewhat vague and only prescence or absence was recordable, despite the fact that equal concentrations were used.

Certain compounds which were present in the methanol-HCl extract were absent from the ethanol extract. These were a group of blue and purple spots running just behind the front in both directions. These may be compounds that were hydrolyzed from a protein partnership by the acid conditions, or they may represent degradation products. The increased concentration in the methanol

extract may also be due to a displacement of equilibrium solubilities by the acid. If the fluorescent compounds are combined and held in some associations, probably with proteins, and if they have only a limited solubility because of these associations, then the acid conditions of the extracting medium could increase their solubilities and hence one would observe greater intensity in the fluorescent spots.

The compounds observed (Table VII) are, with a few exceptions, the same in the two chromosome races (Fig. 11), varying neither in R_f value nor in colour under ultraviolet light. Certain spots are of less constant occurrence, but are occasionally found in both races. The major difference chromatographically concerns a pale green spot with an R_f of 44 x 50 which is present in the tetraploid and which has never been observed in the diploid. Less constant are two other spots--one often found in the diploid at R_f 25 x 0 but never in the tetraploid, and one found occasionally in the tetraploid at R_f 33 x 46 but never in the diploid.

The remaining spots were present in varying proportions in both races, the tetraploid usually producing higher concentrations of the compounds from a given concentration in the extract.

The attempts made to identify the compounds were largely unsuccessful. This may be due to the use of insufficient material during extraction, although up to 12 leaves were used, and the whole of the derived solution was applied to the chromatograph. The acid nature of the solution may have rendered the compounds insensitive to the test chemicals, although it would be expected in such a case that the fluorescent properties of the spots would be different from

Key to Table VII

Colours	Frequency
bl - blue	occasional - (1-40%)
br - brown	common - (40-75%)
g - green	constant - (90-100%)
p - purple	
wh - white	
y - yellow	

Table VII. Chromatographic Results.

Spot #	$R_{f_1}(\pm 10\%)$	$R_{f_2}(\pm 10\%)$	Colour	Frequency
1	0.16	0	br-y	occasional
2	0.25	0	br-y	occasional in 2n
3	0.33	0	wh-y	common
4	0.48	0	wh-y	common
5	0.90	0	r	constant
6	0.35	0.09	g	occasional
7	0.56	0.12	g	constant
8	0.28	0.24	br	constant
9	0.44	0.25	y-g	constant
10	0.58	0.27	g	constant
11	0.80	0.23	bl	constant
12	0.18	0.37	y-g	constant
13	0.13	0.43	br	occasional
14	0.33	0.46	g	occasional in 4n
15	0.44	0.50	g	constant in 4n
16	0.26	0.57	g	constant

Table VII. Chromatographic Results. (continued)

17	0.47	0.58	bl	constant
18	0.57	0.61	p	common
19	0.71	0.51-0.75	p	common
20	0.87	0.64	p	constant
21	0.14	0.84	g	occasional
22	0.22	0.78	g-br	constant
23	0.46	0.82	br-wh	occasional
24	0.60	0.83	p	constant
25	0.79	0.84	p	constant

those prepared from an ethanol extract. As noted above, this is not found. It may be that the chemicals used in the identification tests do not react with all the members of the class of compounds which they are used to detect. Two of the spots, number 8 (R_f 28 x 24) and number 9 (R_f 44 x 25) gave faintly positive results with p-nitrobenzendiazonium tetrafluoroborate indicating that these compounds are probably phenolics.

The evidence from chromatography thus favours the view that the tetraploid race originated through autopolyploidy from the diploid, and that only a little chemical evolution has occurred in either race since their divergence. With regard to the first point, it should be mentioned that chromatographs of Viola selkirkii, V. rugulosa, V. nephrophila and V. palustris are quite dissimilar in pattern (Figs. 12 and 13). Hence the similarity of the two races is not due to a generic uniformity of chromatographic properties. The differences although not marked are consistent and indicate that

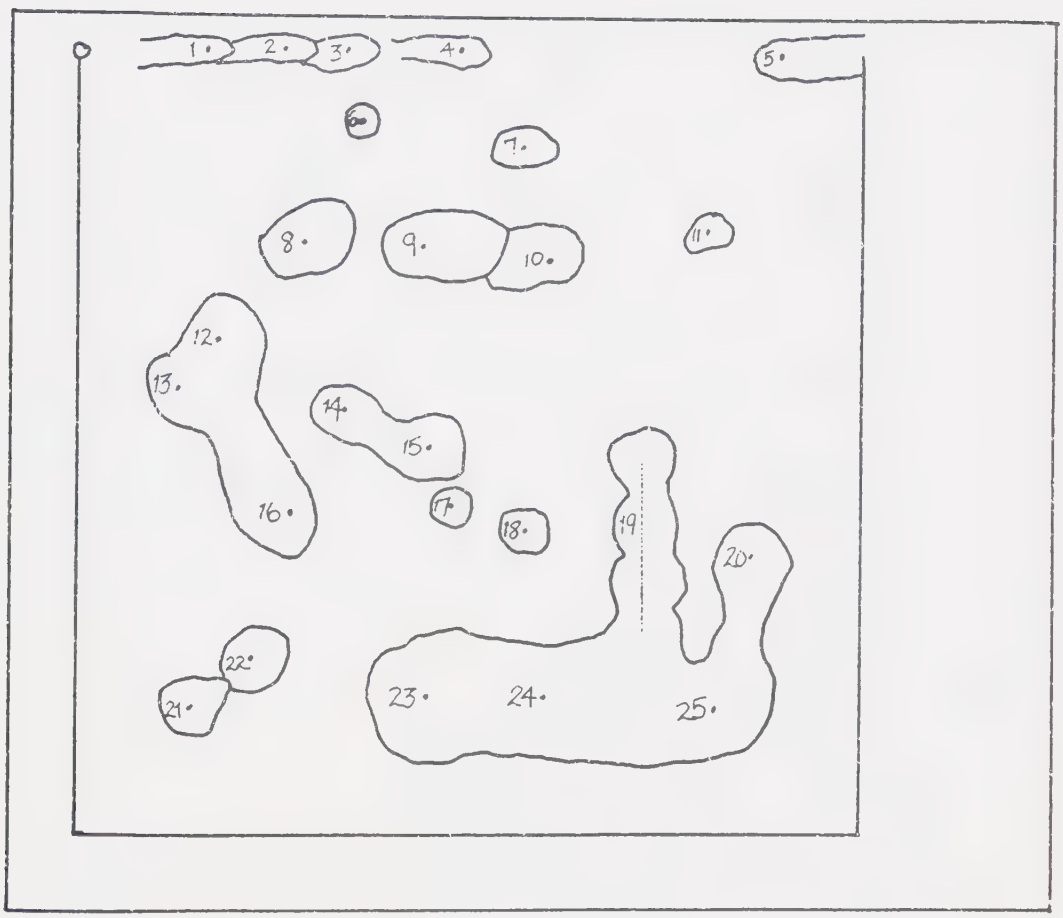
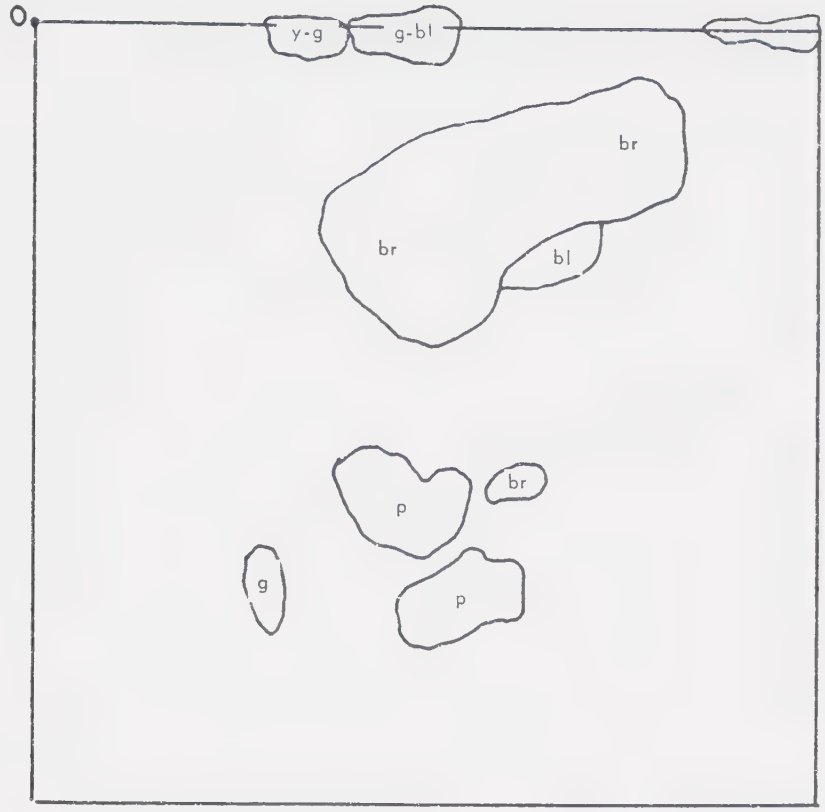


Fig. 11. Generalized paper chromatograph of Viola adunca.

(see Table VII)

V. RUGULOSA



V. SELKIRKII

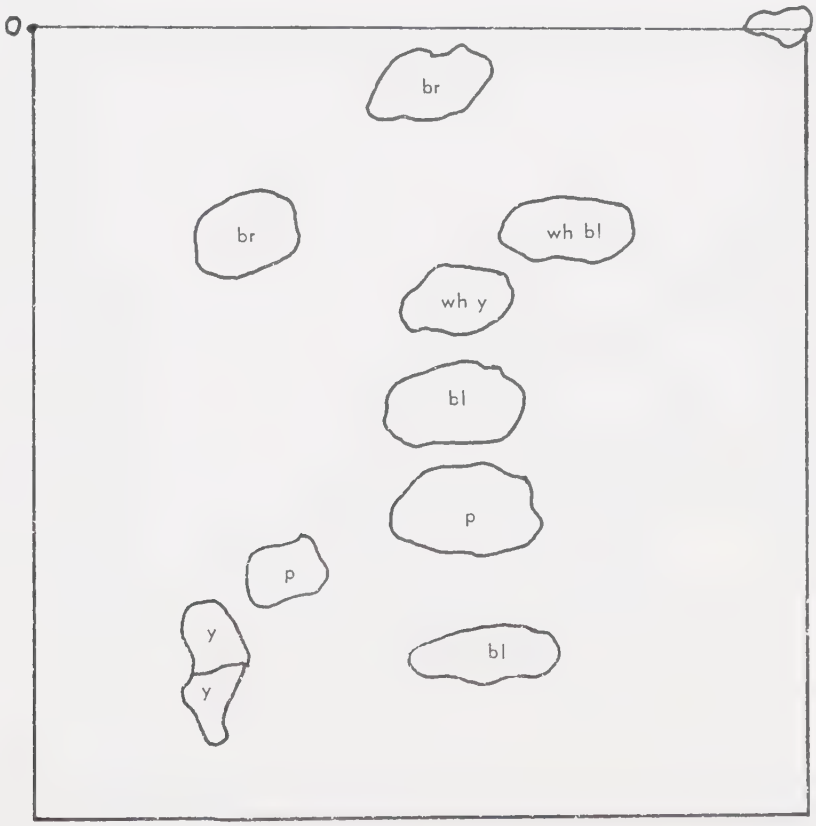
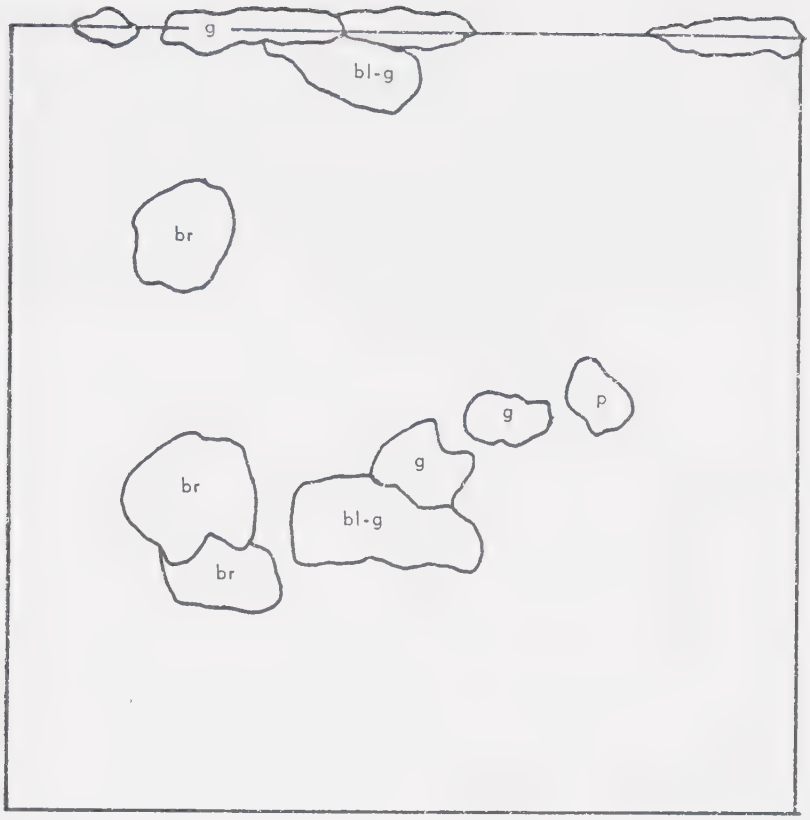


Fig. 12. Paper chromatographic patterns of Viola rugulosa and V. selkirkii.

V. PALUSTRIS



V. NEPHROPHILA

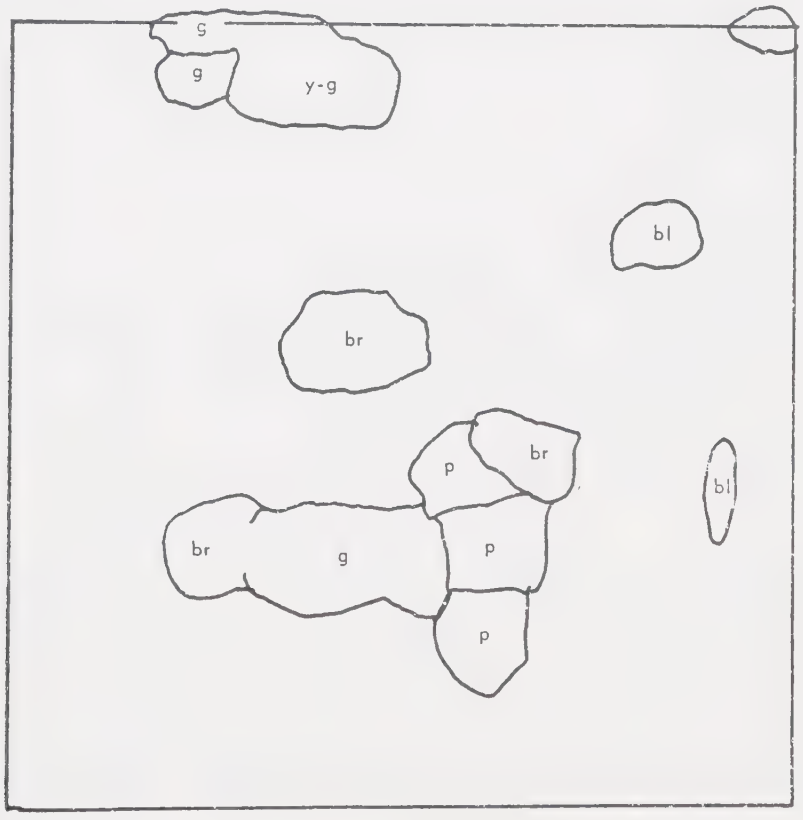


Fig. 13. Paper chromatographic patterns of Viola palustris and V. nephrophila.

there has been a separation of evolutionary lines.

Thin layer chromatography

Thin layer chromatography was not adopted as the primary chromatographic method because the results did not add any information to that available from paper chromatography, and because the latter process was beset with fewer problems.

Of the two media, cellulose proved superior to the silica gel for the latter attached poorly to the glass and broke into flakes as the solvent rose through it. Calcium sulphate was added to the gel-water solution but did not greatly improve the results.

A somewhat similar problem was encountered with the cellulose preparations. As the solvent advanced, it carried a small amount of the cellulose with it, and thus the first front would become marked by a line of more thickly deposited cellulose. This line then interfered with the advance of the second solvent, causing a curved second front to develop, and lowering the R_{f_2}'s of compounds with high R_{f_1}'s, as well as causing streaking of them. However, if the thickness of the cellulose layer was small then the problem was not severe, and affected only 10-15% of the spots that developed. It will be noticed that the chromatographic pattern (Fig. 14) is different from that found when paper is used, although there is a relationship between the two.

Resolution was not improved compared to the paper chromatography, and in many cases it was necessary to apply two to three times as much extracted material in order to demonstrate the occurrence of many spots. The TLC method was faster than the paper

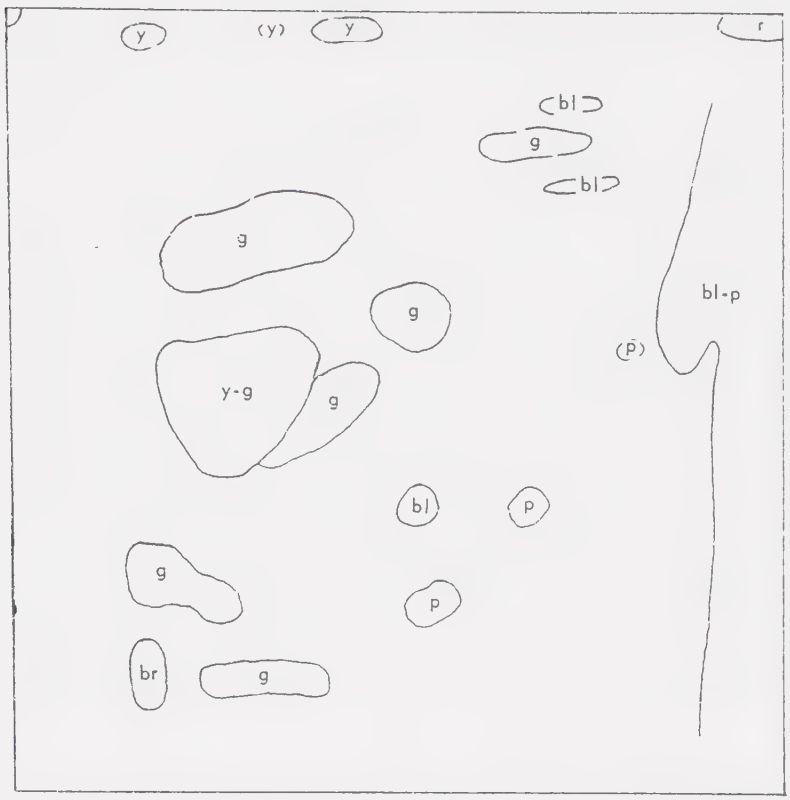


Fig. 14. Thin layer chromatographic pattern of Viola adunca.

chromatography, for a TLC run took about six hours in the first direction, and two hours in the second. The best of the TLC plates prepared suggested that the chromatographic properties of the leaves of the diploids and tetraploids were strongly similar, and the relative difficulty of producing reproducible plates led to the choice of paper chromatography over TLC as a method of analysis.

Distribution

It can be seen from the map (Fig. 15) that the distribution patterns of the two races are markedly different. The tetraploid occupies a more northern area, arching from central and southwestern British Columbia along the mountain valleys and the foothills, across the boreal zone of Alberta and Saskatchewan, and then south towards southeastern Saskatchewan and south-central Manitoba. Diploid material collected on the Ontario peninsula implies that somewhere between Sidney, Manitoba and southern Ontario the two races meet again.

The geographical distinctness of the two races was a striking feature during their collection, for by travelling some 50 miles or so one would pass from a region of uniformly tetraploid plants to one in which only diploids were found. This was the case in southwestern Alberta, where the most intensive collecting was done. Hayman (1960) describes a similar case of exclusiveness in the chromosome races of Themeda australis R.Br. The unmixed nature of the populations throughout their studied ranges provides a tool for predicting the path of the boundary line in other parts of the continent from the collection of a relatively few specimens.

Fig. 15. Collection sites in the Alberta region of populations of Viola adunca.

The single instance of a population of one chromosome number occurring within the range of the other is that of the Cypress Hills in southeastern Alberta and southwestern Saskatchewan. Here the tetraploid race occurs and is probably more abundant than the diploid race, which also occurs there and which surrounds the Hills. The possible significance of this locality will be considered below.

The morphologically distinguishing features outlined earlier have been used to extend the map of the race distributions. Herbarium specimens from Alaska, British Columbia, Washington, Oregon, California, the central western United States, New York, Quebec and New Brunswick have been examined, and a distribution map for the continent based on the morphological investigations appears in Figure 16. This map can be regarded only as a guide to the distributional patterns of the chromosome races. However, if the segregation of the two races is as pronounced elsewhere as it is in Alberta, then the range extensions based on morphology are justified. If that is the case, then almost all of the Viola adunca populations of the United States are diploid, as are those of the Maritimes, Quebec and southern Ontario, as well as those of western British Columbia and Alaska. These results agree with the few reported chromosome counts from these areas (Table IV). Plants from the Northwest Territories and southern Manitoba on the other hand have tetraploid morphological features.

The following is a list of specimens which, except as noted, bear the morphological features of the diploid race. These specimens have a style head with an abundance of long projections, and in many cases the longer pubescence of the diploid race. For approximately

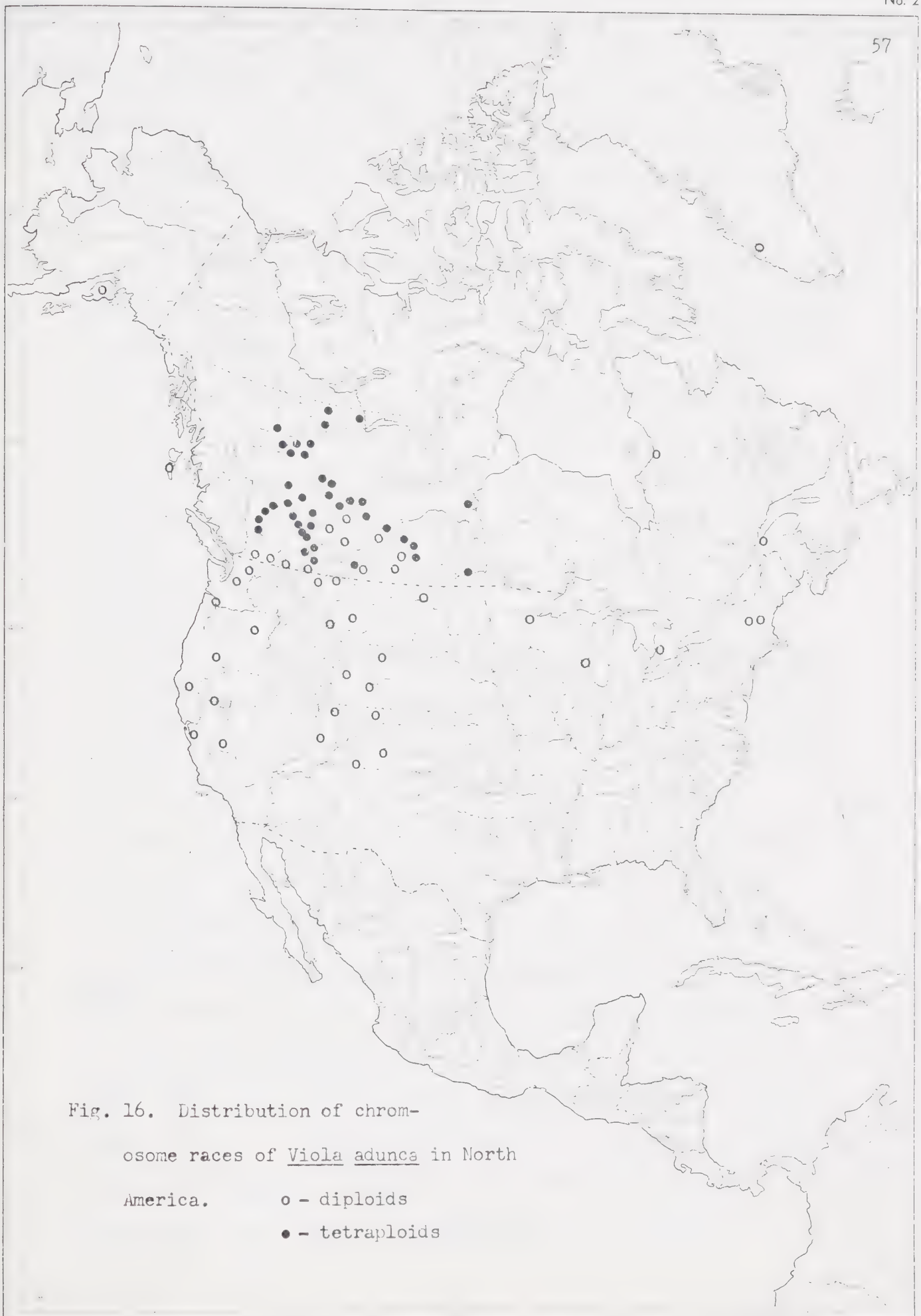


Fig. 16. Distribution of chromosome races of Viola adunca in North America. o - diploids
• - tetraploids

Prepared by Henry M. Leppard

twenty percent of the specimens the length of the guard cells was measured as well, and fell within the range typical of the diploid race. Numbers lacking a collector's name before them are accession numbers.

Alaska: Coopers Landing, Anderson 6904 (GH); Kenai Peninsula, Beaman 589 (GH). Northwest Territories: Fort Smith, 69637 (COLO), tetraploid. British Columbia: Mile 4 Alaska Highway, 94049 (DS), tetraploid; Queen Charlotte Islands, 604245 (DS), 232004 (COLO); Vancouver Island, 143108 (DS); Harrison, Spreadborough s.n. (GH); Dawson Creek, Raup and Abbe 3528 (GH) tetraploid. Saskatchewan: southernmost Saskatchewan, 202200 (COLO). Manitoba: North end of L Winnipeg, Scoggan 4176 (GH) tetraploid. Ontario: Temagami Forest Reserve, Watson 6904 (GH). Quebec. East coast of Hudson Bay 77^{o} x 56^{o}, 118534 (COLO); Rimouski Co, Rousseau 31000 (GH); Vercheres Co, Victorin and Rolland-Germain 34116 (GH). New Hampshire: West of Lebreron, Kennedy 40 (GH); Mt Monadrock, Robinson s.n. May 29/1897 (GH). Vermont: Salisbury, s.c. May 13/1900 (GH). Wisconsin: Baron Co, Fasset 15670 (GH); Montesano, 42400 (COLO). Minnesota: Beltrami Co, 97824 (COLO). North Dakota: Butte, s.c. June 6/09 (GH). Montana: Madison Co, Nelson 5433 (GH); Bozeman Co, Hutton s.n. (GH); Beaverhead Co, Hitchcock and Muhlick 12751 (GH), 159833 (COLO). Idaho: Custer Co, Hitchcock and Muhlick 9401 (GH); Lemhi Co, Payson 1861 (GH), 42429 (COLO), 42403 (COLO). Washington: 15183 (COLO), 86384 (COLO); Pullman, 20328 (COLO); Mason Co, 21081 (COLO): Whitman Co, 21078 (COLO). Oregon: Falls City, Nelson 1130 (GH); Dallas, Harford and Dunn s.n. (GH); Portland, Drake and Dickson s.n. (GH); Grant Co, Henderson 5529 (GH). Wyoming:

Yellowstone National Park, 115597 (DS); Lincoln Co, Payson 3338 (GH); Albany Co, Porter 3071 (GH); Pale Creek, Nelson 146 (GH); Ashley National Forest, Porter and Miller 5950 (GH). California: North Lake Co, 164834 (UC); Humboldt Co, 176962 and 164813 (UC); San Bernardino Co, M121561, 838099 (UC); Santa Cruz Co, M121562 (UC); Siskiyou Co, 164289, 581515, 909368, 164273 (UC), 494550, 249237 (DS), Heller 12086 (GH); San Francisco, M300825, 35282 (UC); Monterey Co, 19266 (UC); Pasadena, 140886 (UC); Madera, 526661 (UC); Tuolumne, 581506 (UC), 475010, 259169, 85580, 445251, 247169 (DS), Porter 700 (GH); Sonoma, 19264 (UC); Sequoia National Park, 309926 (DS); Yosemite Valley, 31160 (UC); San Marco Co, 110064 (UC); Modoc, 345495 (DS); Alexander and Kellogg 4820 (GH); Fresno Co, 395044 (DS); Castle Lake, 277317 (DS); Fort Bragg, 106688 (DS), 81443 (COLO); San Bruno Hills, Baker 1880 (GH); Glenn Co, Ownbey 1726 (GH); Sessien, Copeland 3721 (GH). Utah: Provo, Goodding 1118 (GH); Duchesne Co, 100213 (COLO); Uintah, 169571 (UC). Colorado: South Park, Hughes s.n. (GH), 42392, 42396, 42386, 42387, 42384, 42377 (COLO); Park Co, 184648 (COLO); Larimer Co, 42421 (COLO); Gilpin Co, 42515, 42418, 42411 (COLO); Garfield, 190417 (COLO); Routt. 200973 (COLO); Daggett, 77173 (COLO); Tolland 42420 (COLO); Sulfur Spring, 42397 (COLO); Silverton, 42378, 42393 (COLO); La Veta Pass, 42427 (COLO).

In considering this distribution pattern, it is important to remember that western Canada was almost completely covered by the last glaciation (Flint 1971), only a peninsula extending north from near Waterton National Park being left untouched (Fig. 17). Some speculation exists of other refugia having occurred along the

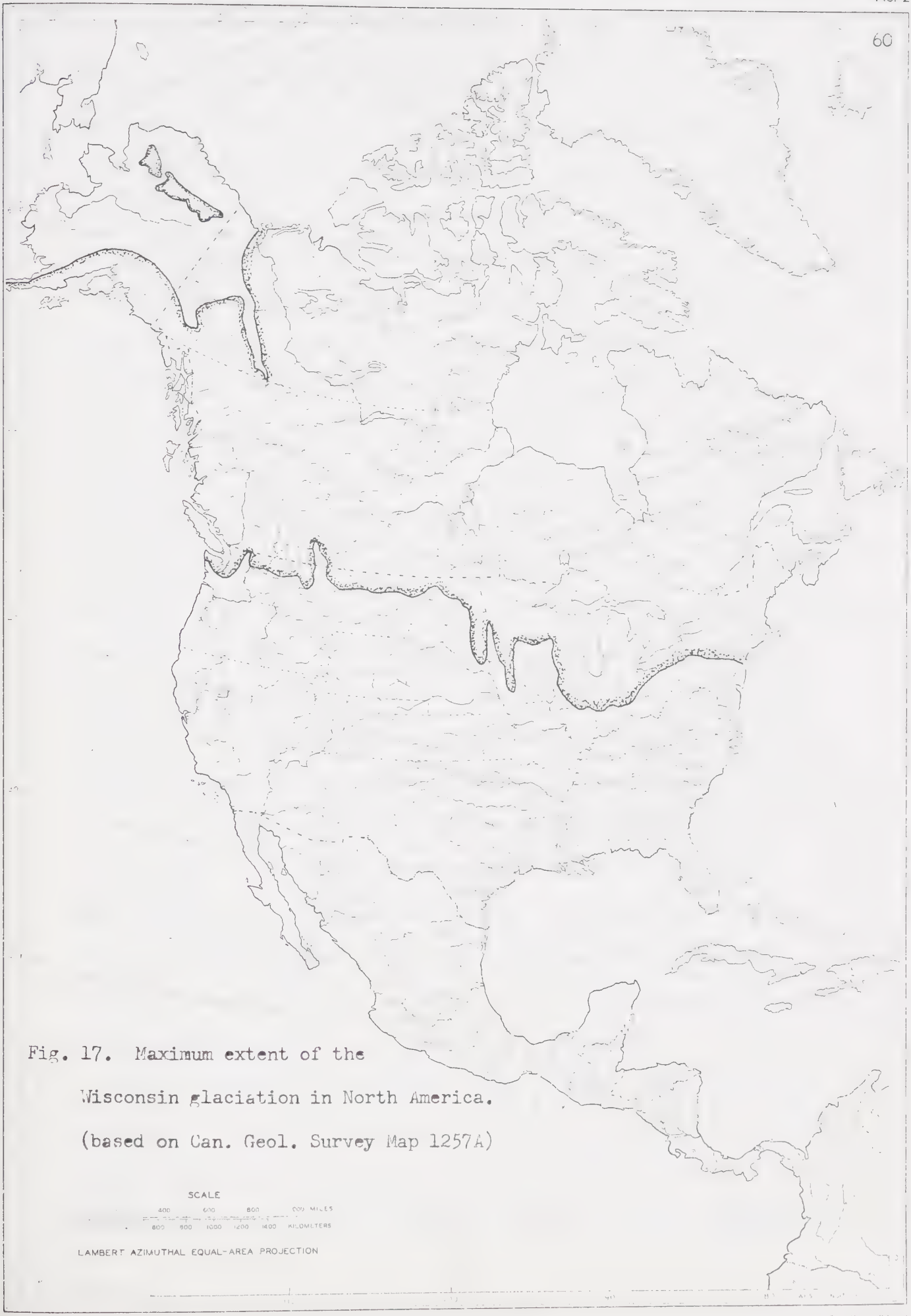


Fig. 17. Maximum extent of the Wisconsin glaciation in North America. (based on Can. Geol. Survey Map 1257A)

foothills, but there is a shortage of critical data. If it is accepted that the polyploids arose in the manner that Stebbins has proposed (see introduction) i.e. in periglacial areas and areas recently vacated by the ice sheet, then much of the present distribution of this race is explained. Thus if the tetraploid originated in the unglaciated Waterton peninsula or in the periglacial zone south of the ice front, it could have spread northward easily into the unoccupied niches created as the glacier retreated. If there were, in addition, some differential in ecological responses between the tetraploid and the diploid, such that the tetraploid was better suited to cooler, forested regions, then it would spread north and east quickly but need not have displaced the diploids growing in the forest to the south and west. These would be established populations, and infiltration of them by the tetraploid would be a slow process. The western and Appalachian diploids would invade the newly opened territories to the north of them successfully because of the absence of the tetraploid. The Alaskan and Yukon refugia may have harboured diploids which would spread south subsequent to the retreat of the ice. The Cypress Hills may have received tetraploids at an early point during the retreat, and then have been colonized by diploids from the Prairie to the south as the region recovered its former climate. On the other hand, there is some evidence that the Cypress Hills were unglaciated. In that case the diploids' presence there would be simply due to persistence, and it would be the tetraploids which last invaded. To reiterate, two postulates--firstly a northern frontier place of origin for the tetraploid, and secondly an ecological advantage in forested regions--would, if correct, explain the

observed distributions of the two races.

Barber (1970) speculates on a possible biochemical explanation for the frequently greater tolerance of polyploids over their diploids with respect to invasion of newly available habitats. Barber (l.c.) contends that the hardiness of autopolyploids is similar to the phenomenon of heterosis. The different products of alleles, he believes, confer on the plant a "biochemical versatility" which he suggests has been demonstrated in corn where a hybrid variety shows less sensitivity to temperature shocks than do its parent varieties (McWilliam and Griffing 1965). He goes on to speculate that the presence of several alleles of a gene would result in the production of what he calls "hybrid enzymes." If most enzymes are composed of oligomers of the primary gene product (much in the fashion that mammalian haemoglobin is composed of four chains) then the enzymes compounded from the products of different alleles may have different properties. Barber points to the wider range of activity in regard to pH and some inhibitors that has been demonstrated for a hybrid enzyme in maize (Schwartz and Laughner 1968) and to some electrophoretic work of his own (Barber *et al*. 1968) which tend to support his idea of the hybrid enzyme with different properties. These new enzymes may enable the population to develop normally in ecological situations which formerly had been closed to it.

This provocative idea finds some support in the literature for there are many examples of species in which heterozygotes are at a relative advantage under stress as compared to homozygotes (Lewis 1955, Adams and Shank 1959, Clausen and Hiesey 1958, McWilliam and Griffing 1965). On the other hand in several species examined, the

vigour of hybrids under various conditions has been found to only equal or to actually be less than the vigour of the parents (Jinks and Mather 1955, Smith and Daly 1959). Furthermore what little is known about the hybridization of enzymes has been learned from Drosophila studies so that Barber's hypothesis must be viewed with caution.

Attempts at correlating the distribution patterns with contemporary environmental factors have met with some success. The species is found in a rather wide range of habitats. Specimens have been collected from crevasses in rocky slopes at or above the alpine treeline, from shaded and open mixed forest, from grassy meadows, and and from shrubby prairie hollows. A common factor in all these habitats seems to be a minimum amount of shading--in all cases some protection is afforded the species from full exposure to the sun. Whether it is the shade itself or the concomitant higher local humidity, or both factors together, that promotes growth is unknown. Soil type varies from very sandy loam to rich black loam to little more than fragmented shale.

However, the map (Fig. 18) of the soil types found in the Prairies shows that the line delimiting the Prairie soils as opposed to the Parkland and Timber soils runs through roughly the same areas as the line separating the two chromosome races. In some areas, particularly the Parkland zone, man's activities have changed the type of prevalent vegetation to something other than that which typifies the undisturbed situation. This fact may explain the somewhat more northerly course of the race boundary as compared to that of the two soil types.

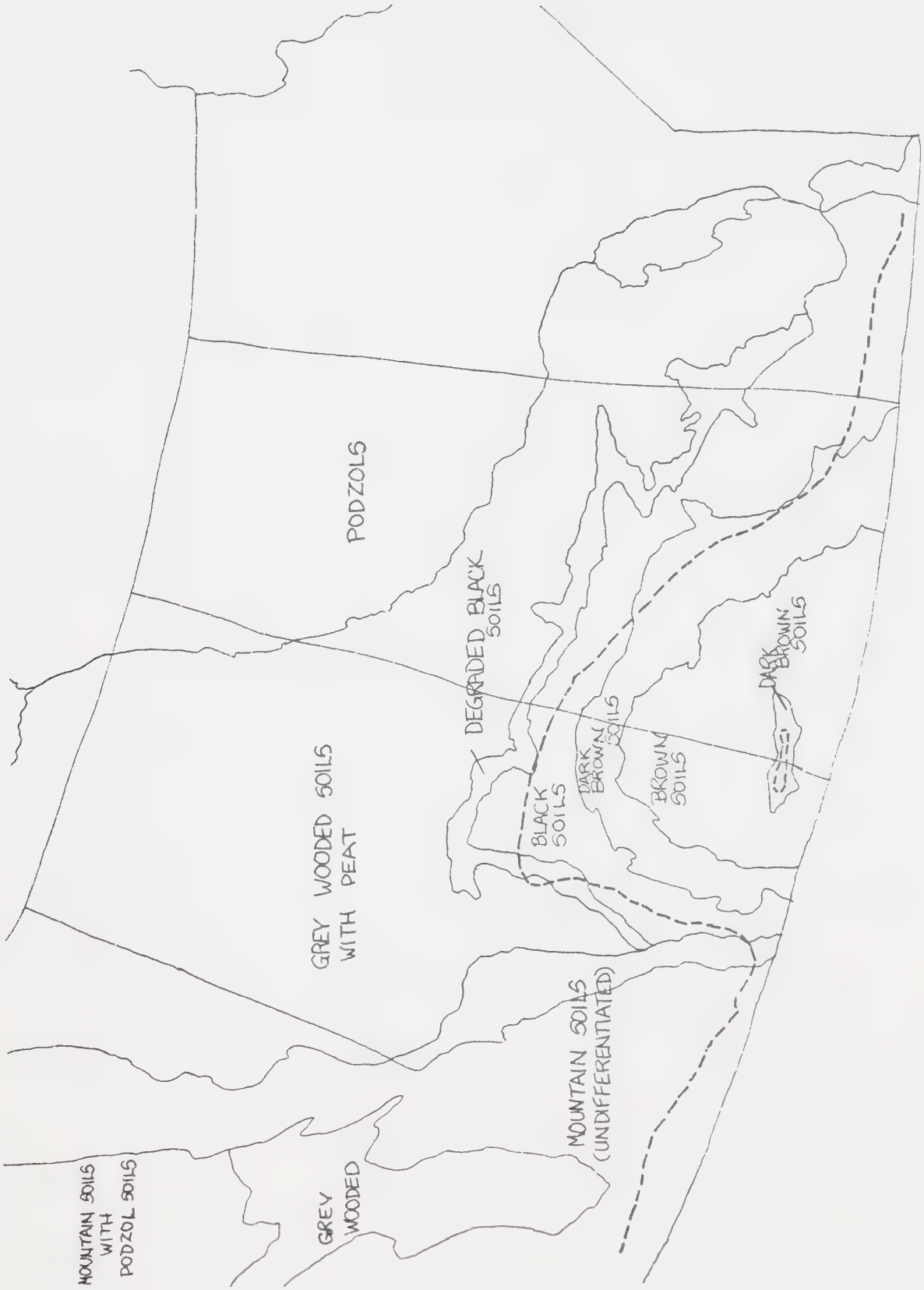


Fig. 18. Soil types of the Prairies. Dashed line indicates common border of the two chromosome races in Viola adunca.

The occurrence in the Cypress Hills of the tetraploid race is consistent with the parkland or forested nature of these areas. The soil type there is described as much like that found between the true prairie soil and the soil of treed regions.

A graph of the April and May isotherms for this region (Fig. 19) shows that the 40° F isotherm for April and to a lesser extent the 50° F isotherm for May both parallel the border line between the two races. The map of the isomers of the number of days per year above 60° F similarly separates the two regions (Fig. 20) along the 150 days line.

These parameters taken together point to the existence of a climatic boundary near the chromosomal border, and provide a basis for the belief that the two races have adapted to somewhat different environmental pressures.

Of the above factors, it seems likely that soil type is the most important, for an explanation of the distribution patterns based primarily on kind of soil accounts for the occurrence of tetraploids in the Cypress Hills. The other factors do not suggest that tetraploids might be expected there. It obviously cannot be concluded that the Parkland-Timber soil types demand the tetraploid's physiology exclusively, for these soils are found in Washington, Oregon and the valleys of British Columbia where the diploid grows quite well and where the tetraploid is not found. On the other hand the tetraploid does not occur in the Prairie soil type.

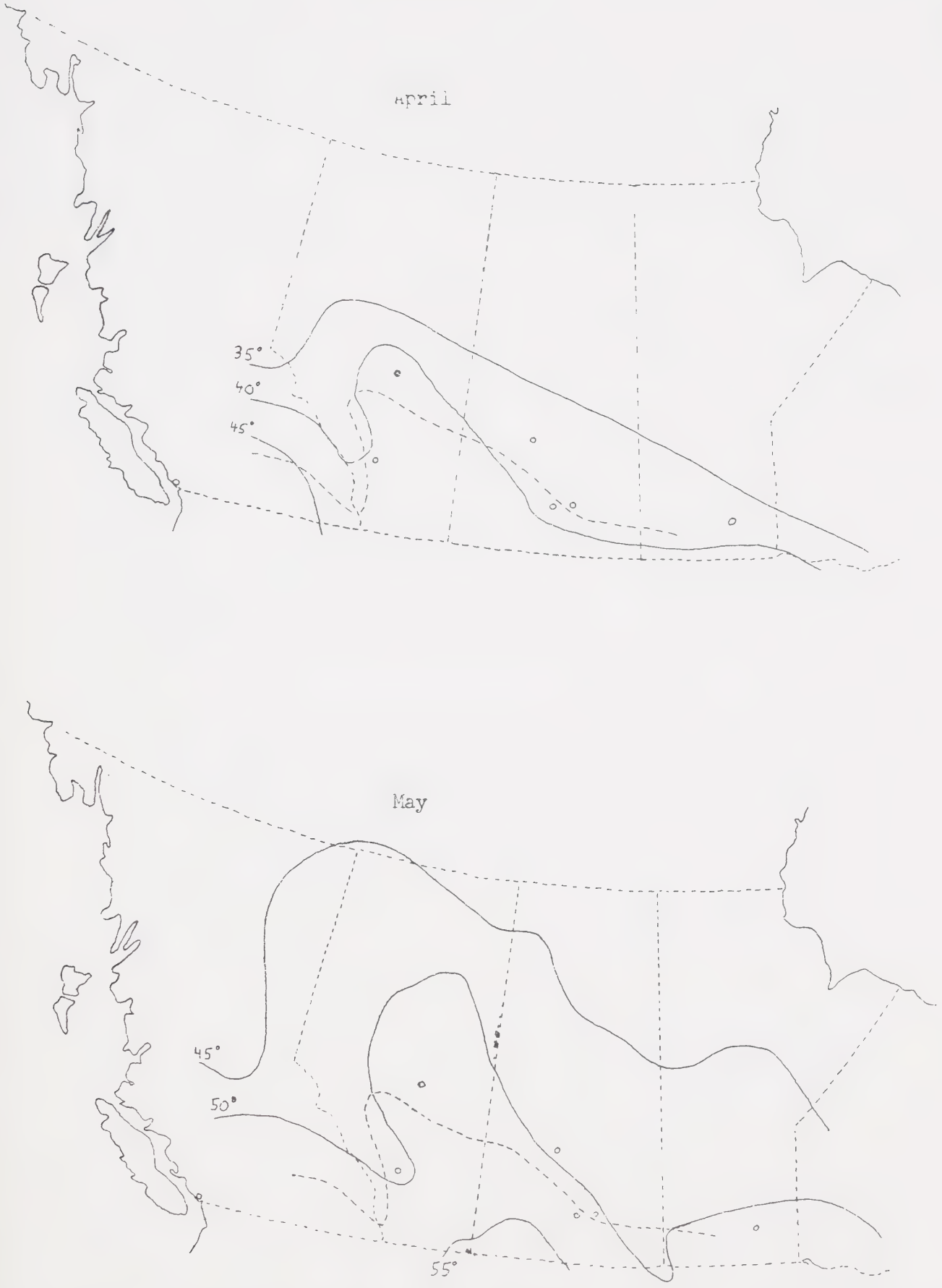


Fig. 19. Spring isotherms of the Prairies. Dashed line indicates common border of chromosome races in Viola adunca. (based on Atlas of Canada 1915)

Fig. 20. Number of days above 60° F. Dashed line indicates common border of chromosome races in Viola adunca. (based on Atlas of Canada 1915)

Dispersal

The dispersal mechanism of the seeds of Viola adunca creates the necessity of explaining the present distributions in terms of animal vectors. The capsule of the species splits along its three sutures into three valves, and as the walls of these dry out the seeds are squeezed between them and eventually shot away for distances of 6 to 12 feet. If the distance that seeds of this species could have travelled in the less than 20,000 years since the retreat of the last glacier is calculated, on the basis of a 10'/year advance, then a figure of less than 40 miles is obtained. Since the last glacier extended to roughly the 49th parallel, it is clear that some other mechanism is at work besides the species' explosive capsule, for V. adunca is found beyond the 60th parallel, some 750 miles to the north.

After flowering the stems often recurve, hiding the capsules amongst the leaves, a habit which in other species of this genus is associated with dispersal by ants (V. odorata, V. hirta, V. tricolor), and an oil-body is commonly also found which attracts the ants. This oil-body, which is white in most cases, may be the product of the funicle or the strophiole. The white funicle is persistent on the seeds of V. adunca but this raphe is not obviously oily. Specimens are not infrequently found associated with ant colonies, and may be locally dispersed by ants. However this means of spreading is also inadequate to explain the distribution of the species in glaciated regions. Dispersal by birds is excluded as a probable method because of the habit of the fruits and their green colouration. Dispersal by mammals on the other hand is more likely in view of the

multitude of small forest-floor mammals and their feeding habits. As well, deer species may play a role, for in Europe the intact seeds of V. tricolor and V. biflora have been recovered (Ridley 1930) from the excreta of the fallow deer (Cervus dama) and the reindeer (Rangifer tarandus). The deer and the small animals of western North America might be expected to have extended their ranges and that of V. adunca by invading new regions as they became free from the glaciers. Dispersal by such animal vectors would therefore explain the deep penetration of V. adunca into glaciated western North America. Such dispersal methods would also maintain, or at least not constantly threaten, the distinctness of the distribution of the two chromosome races, whereas long-range dispersal as would be done by birds would probably disrupt the present pattern.

A feeding experiment conducted using lab mice failed because of technical difficulties (concerning maintenance mainly, but also due to the small gullet of these animals), but did demonstrate that the capsules are considered edible by such animals. A further test using native forest mammals will be made when these animals are available.

Interracial breeding

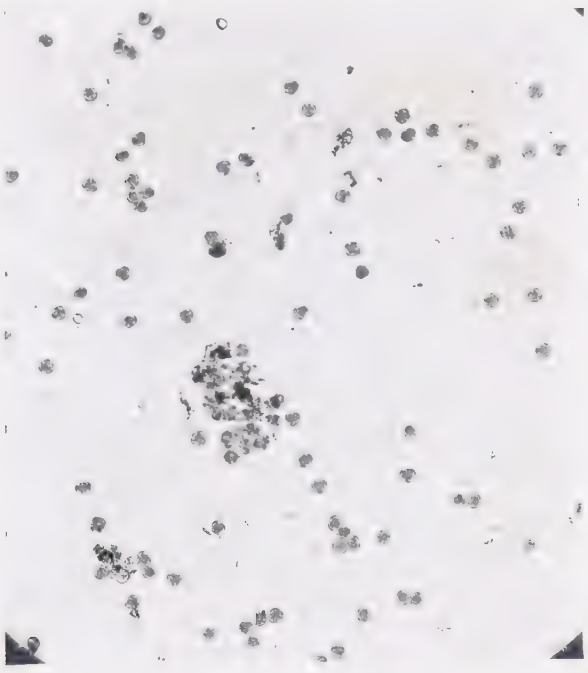
Interbreeding between the two races is known to go on for triploid specimens have been collected. These plants have been found only in the region in which the two races contact one another, and they do not seem to be common even along this line. They grow vigorously and do not differ in vegetative features from the diploids and the tetraploids. However, both types of flowers are found in a

modified form. Cleistogamous flower buds begin to develop but do not reach maturity and do not set seeds. The aborted flowers soon shrivel on the plant. The chasmogamous flowers that have been observed suffer a similar fate. In their case the buds are smaller than normal, and, if they begin to unfold, the petals are much reduced in size so that the whole flower is less than 7 mm long. In most cases, however, the chasmogamous flowers do not develop at all. Pollen viability, as judged by a lacto-phenol staining test, is high in the diploid and in the tetraploid, but low in the triploid (Plate III). In other words the F_1 generation of a diploid X tetraploid cross is viable but sterile, and thus the two parent races are reproductively isolated.

The significance of this observation to proponents of the "biological species definition" is apparent. According to this definition both the chromosome races should be recognized as distinct species simply by virtue of their genetic isolation. On the other hand the close morphological and chromatographic similarity of the two taxa are facts which would be held up by adherents of the "morphological species definition" as indicating that the two races should be maintained in one species. The view taken here is essentially the latter, and is based on a consideration of the autotetraploidy and allopatry which obtain in this case, as well as pressures of practicality and a belief that reproductive isolation in such closely related taxa as these is a difficult concept. This belief is based on the reflection that reproductive isolation occurs along a continuum. Thus during the time of divergence, a mere decrease in the amount of fertility of interracial as compared to intraracial

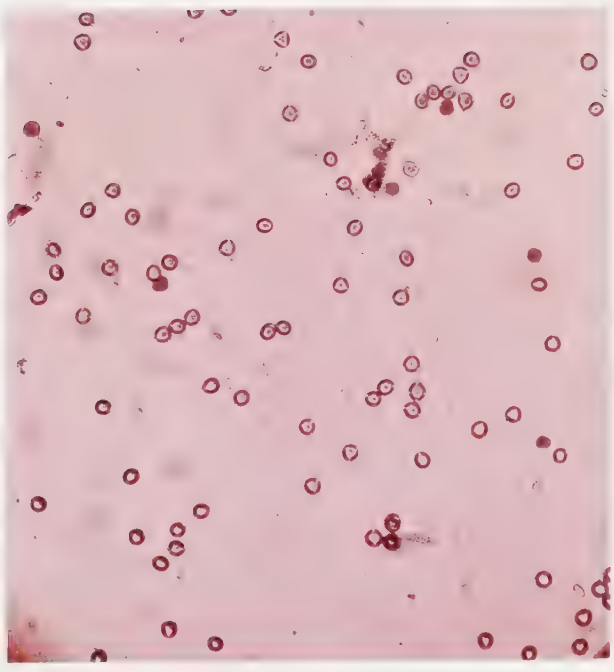
Plate III. Pollen grains stained in lacto-phenol.

Diploid



Triploid

Tetraploid



crosses would permit the evolution of two ecologically different groups. Yet these emerging races would be only partially isolated, and would not be considered "biological species." Should the genetic barriers then become more complete, a decision on when to recognize "biological species" would involve either the use of an arbitrary limit to the percentage of infertility or a demonstration that isolation is 100%. The former alternative is obviously unsatisfactory, and the latter is almost impossible to demonstrate. In this case, the small number of triploids studied is inadequate basis for such a generalization, and furthermore the recreation of the tetraploid from the diploid is not impossible, and hence genes may flow from the diploid is not impossible, and hence genes may flow from the diploid to the tetraploid in this manner. If only because of this possibility it would seem that recognition of the tetraploid as a "biological species" is unjustified.

The proper level of recognition therefore appears to be that of subspecies. It is currently held that subspecies are comprised of geographically distinct populations which are morphologically separable on some grounds (Du Rietz 1930, Babcock 1947, Camp and Gilley 1947, Meikle 1957). This definition avoids the difficulties of reproductive isolation, and it would seem, does as much to reflect the evolution that is in progress as the "biological species definition" attempts to do. That is, the fact that there are two entities present is indicated. At the same time the origin of the tetraploid by autotetraploidy is not obscured by its complete separation from the diploid. Furthermore, and perhaps most importantly, the practical problem of distinguishing between the two races does not have to be

solved except by those specialists interested in detailed analysis.

The rather conservative position adopted here is in sympathy with the view of Davis and Heywood (1963), and with Gershoy's opinion on species limits in the genus (Gershoy 1934). In contrast is the view of Valentine (1950), and Valentine and Love (1958) who believe that a slight morphological difference between the members of a polyploid series, especially when coupled with allopatry, is ample grounds for the recognition of separate species. Valentine recommends that if such species should be unidentifiable under some conditions, then they be included in aggregate species for convenience, leaving to those doing critical work in ecology or phytogeography the task of exact species determination. However, the general arguments presented above apply to the specific opinions of this student of Viola.

Conclusion

In conclusion the results of the investigation of the chromosome races of Viola adunca can be stated briefly as follows. Morphologically the diploid and tetraploid are similar, but separable on characters of the style, the pubescence, and the guard cell and pollen size. Chromatographically the two races are quite similar, differing only slightly in pattern. The relationship between them is concluded to be one of autopolyploidy. Reproductively the races appear to be strongly isolated. Geographically they are distinct from one another, as a result, it is suggested, of historical and ecological factors. The two taxa are therefore considered to merit recognition as individual subspecies, and the subspecific epithet

albertae is proposed for the tetraploid race.

Five other subspecies have been named in this species. Four of these--V. adunca ssp. oxyceras Piper, V. adunca ssp. ashtonae M.S. Baker, V. adunca ssp. radicosa M.S. Baker and V. adunca ssp. typica M.S. Baker (which should be named V. adunca ssp. adunca according to Articles 24 and 26 of the International Code of Botanical Nomenclature)--have been cytologically examined (Clausen, Keck and Hiesey 1940) and found to be diploid. The fifth subspecies, V. adunca ssp. uncinulata Applegate, occurs within the range of the diploid specimens (Applegate 1939), and therefore has been rejected as a possible synonym for the tetraploid subspecies. No previous taxonomic recognition has been accorded the western Canadian populations of V. adunca. Hence the new epithet albertae has been coined, and there appears to be little likelihood that it will prove to be a later synonym.

Description of V. adunca ssp. albertae.

VIOLA ADUNCA subspecies ALBERTAE McPherson, ssp. nov*.
Pubescentia foliis 0.22 mm longa. Capitulum style glabrum vel habens projecturae conicae, 1/10 latitudae capitule styli. Cellulae accessoriae 27-35 u longae. Pollen 42-49 u longae. Numerus chromosomorum 2n=40.

VIOLA ADUNCA subspecies ALBERTAE McPherson.
Pubescence of leaves to 0.22 mm long. Head of style smooth or bearing conical projections up to 1/10 the width of style head. Guard cells 27-35 u long. Pollen 42-49 u long. Chromosome number

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*To be validly published later.

2n=40. The holotype of this subspecies was collected in mixed pine and poplar forest 30 miles southwest of Edmonton on June 4, 1972, and has been numbered McPherson 578. It has been deposited in the University of Alberta Herbarium (ALTA).

Distribution: Central British Columbia and foothills of Alberta to parkland and forests of Alberta, Saskatchewan, Manitoba, and the Northwest Territories.

Both chromosome races fit the species description given in the introduction, but in the case of the diploid the following information could be added to complete its characterization. The ornamentation of the style apex varies from a glabrous condition to one in which large prominent projections up to one-sixth the width of the style apex are found. Leaf pubescence varies from 0.00 to 0.33 mm in length. Length of the guard cells average 26.7 microns, and the average length of mature pollen grains is 39.9 microns. The diploid is found throughout the United States and eastern Canada, as well as on the southern Prairies and in southern and western British Columbia.

Key to the subspecies in Viola adunca (largely after Baker 1935b).

1. Style apex smooth to very rough, projections to 1/6 width of style head; leaf pubescence 0.00 - 0.33 mm long; guard cells 27 u long; pollen grains 40 u long; southern and western British Columbia, southern Prairies, eastern Canada, and the United States.....2.
   2. Capsules not notched at apex.....V. adunca ssp. adunca
   2. Capsules notched at apex.....3.
      3. Petals whitish at base; local in N. Colorado.....4.
         4. Flowers pale blue; plants glabrous; leaf bases often truncate.....V. adunca ssp. ashtonae
         4. Flowers purple; plants pubescent; leaf bases cordate .....V. adunca ssp. radicosa
      3. Petals purplish at base; local in S. Oregon .....V. adunca ssp. uncinulata

1. Style apex smooth to slightly rough, projection to 1/10 width of style head; leaf pubescence 0.00 - 0.22 mm long; guard cells 31 u long; pollen grains 45.8 u long; central British Columbia, Alberta foothills, northern Prairies and Northwest Territories .....V. adunca ssp. albertae

REFERENCES

Adams, M.W. and D.B. Shank. 1959. The relationship of heterozygosity to homeostasis in maize hybrids. Genetics 44: 77-786.

Alston, R.E. and B.L. Turner. 1962. New techniques in analysis of complex natural hybridization. Proc. Natl. Acad. Sc. U.S. 48: 130-137.

Alston, R.E. and B.L. Turner. 1963. Biochemical Systematics. Prentice-Hall, New Jersey.

Anderson, E. 1949. Introgressive Hybridisation. New York.

Applegate. 1939. (Title unknown--reference taken from Hitchcock et al 1961). Am. Midl. Nat. 22: 232.

Babcock, E.B. 1947. The Genus Crepis. Part One: The taxonomy, phylogeny and evolution of Crepis. Univ. Calif. Publ. Bot. 21.

Baker, M.S. 1935a. Studies in western violets. I. Sections Chamaemelanium and Nomimium. Madrono 3: 51-57.

Baker, M.S. 1935b. Studies in western violets. II. Madrono 3: 232-239.

Barber, H.N. 1970. Hybridization and the evolution of plants. Taxon 19: 154-160.

Becker, W. 1923. Violae Asiaticae et Australenses. IV. Beih. zum Bot. Centralbl., xl, Abt. 11: 20-68.

Becker, W. 1925. Viola. Die naturlichen Pflanzenfamilien 21: 329-363.

Bowden, W.M. 1940. Diploidy, polyploidy, and winter hardiness relationships in the flowering plants. Amer. Jour. Bot. 27: 357-371.

Brainerd, E. 1904. Hybridism in the genus Viola. Rhodora 6: 213-223.

Brainerd, E. 1906a. Hybridism in the genus Viola. II. Rhodora 8: 6-10.

Brainerd, E. 1906b. Hybridism in the genus Viola. III. Rhodora 8: 49-61.

Brainerd, E. 1921. Violets of North America. Verm. Agr. Exp. Sta. Bull. 224.

Brainerd, E. 1924. Some natural violet hybrids of North America. Verm. Agr. Exp. Sta. Bull. 239.

Britton, N.L. and A.B. Brown. 1913. An Illustrated Flora of the Northern United States, Canada and the British Possessions. Second edition. Charles Scribner's Sons.

Camp, W.H. 1947. Distribution patterns in modern plants and the problems of ancient dispersals. Ecol. Monogr. 17: 159-183.

Camp, W.H. and C.L. Gilly. 1943. The structure and origin of species. Brittonia 4: 323-385.

Clausen, J. 1921. Studies on the collective species Viola tricolor L. Bot. Tidsskr. 37: 206-221.

Clausen, J. 1922. Studies on the collective species Viola tricolor L. II. Bot. Tidsskr. 37: 363-416.

Clausen, J. 1927. Chromosome number and the relationship of species in the genus Viola. Annals of Botany 41: 677-714.

Clausen, J. 1930. Inheritance of variegation and of black flower color in Viola tricolor L. Hereditas 13: 342-356.

Clausen, J. 1951. Stages in the Evolution of Plant Species. Cornell University Press, N.Y.

Clausen, J. 1964. Cytotaxonomy and distributional ecology of western North American violets. Madrono 17: 173-197.

Clausen, J. and W.M. Hiesey. 1958. Experimental studies on the nature of species. IV. Genetic structure of ecological races. Carnegie Inst. Wash. Publ. 615: 1-312.

Clausen, J., D.D. Keck and W.M. Hiesey. 1940. Experimental studies on the nature of species. I. The effect of varied environments on western North American plants. Carnegie Inst. Wash. Publ. 520.

Clausen, J., D.D. Keck and W.M. Hiesey. 1945. Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autoploidy, with examples from the Madiinae. Carnegie Inst. Wash. Publ. 564.

Crawford, D.J. 1970. Systematic studies on Mexican Coreopsis (Compositae). Coreopsis mutica: flavonoid chemistry, chromosome numbers, morphology and hybridization. Brittonia 22: 93-111.

Davis, P.H. and V.H. Heywood. 1963. Principles of Angiosperm Taxonomy. Oliver and Boyd, Edinburgh.

De Wet, J.M.J. and J.R. Harlan. 1972. Chromosome pairing and phylongenetic affinities. Taxon 21: 67-70.

Du Rietz, G.E. 1930. The fundamental units of biological taxonomy. Svensk Bot. Tidskr. 24: 333-428.

Faegri, K. and L. van der Pijl. 1966. The Principles of Pollination Ecology. Pergamon Press, Toronto.

Fernald, M.L. 1950. Viola, in Gray's Manual of Botany. 8th edition. American Book Co., N.Y.

Flint, R.F. 1971. Glacial and Quaternary Geology. John Wiley and Sons Inc., N.Y.

Gershoy, A. 1928. Studies in North American violets. I. General considerations. Verm. Agr. Exp. Sta. Bull. 279.

Gershoy, A. 1934. Studies in North American violets. III. Chromosome numbers and species characters. Verm. Agr. Exp. Sta. Bull. 367.

Hagerup, O. 1931. Uber Polyploidie in Beziehung zu Klima, Okologie und Phylogenie. Hereditas 16: 19-40.

Hagerup, O. 1933. Studies on polyploid ecotypes in Vaccinium uliginosum L. Hereditas 18: 122-128.

Harvey, M.J. 1966. Cytotaxonomic relationships between the European and North American Rostrate violets. New Phytologist 65: 469-476.

Hayman, D.L. 1960. The distribution and cytology of the chromosome races of Themeda australis in southern Australia. Austr. Jour. Bot. 8: 58-68.

Heilborn, O. 1926. Bidrag till violaceerna cytologi. Svensk Bot. Tidskr. 20: 414-419.

Hitchcock, C.L., A. Cronquist, M. Ownbey, and J.W. Thompson. 1961. Vascular Plants of the Pacific Northwest. U. of Washington Press, Seattle.

Hulten, E. 1968. Flora of Alaska and Neighbouring Territories. Stanford U. Press.

Jinks, J.L. and K. Mather. 1955. Genetical control of stability in development. Proc. Roy. Soc. Lond. B. 143: 561-578.

Johnson, A.W. and J.G. Packer. 1965. Polyploidy and environment in artic Alaska. Science 148: 237-239.

Johnson, A.W., J.G. Packer and G. Reese. 1965. Polyploidy, Distribution and Environment. in The Quaternary of the United States. (H.E. Wright and D.G. Frey, Editors). pp. 497-507.

Lanjouw, J. and F.A. Stafleu. 1964. Index Herbariorum. Part I. Regnum Vegetabile 31.

Lewis, D. 1955. Gene interaction, environment and hybrid vigor. Proc. Roy. Soc. Lond. B 144: 178-185.

Love, A. 1950. Taxonomic evaluation of polyploids. Caryologia 3: 263-284.

Love, R.M. and D. Love. 1943. The significance of differences in the distributions of diploids and polyploids. Hereditas 29: 145-163.

Madge, M.A.P. 1929. Spermatogenesis and fertilization in the cleistogamous flower of Viola odorata, var praecox Gregory. Annals of Botany. 545-577.

McWilliam, J.R. and B. Griffing. 1965. Temperature-dependent heterosis in maize. Austr. J. Biol. Sci. 18: 569-583.

Meikle, R.D. 1957. What is the subspecies? Taxon 6: 102-105.

Melchior, H. 1925. Violaceae. Die naturlichen Pflanzenfamilien 21: 363-376.

Miyaji, Y. 1913. Untersuchungen uber die Chromosomenzahlen bei einigen Viola-Arten. Bot. Magaz. 27, 1913.

Moss, E.H. 1959. Flora of Alberta. University of Toronto Press.

Muntzing, A. 1936. The evolutionary significance of autopolyploidy. Hereditas 21: 263-378.

Packer, J.G. 1964. Chromosme numbers and taxonomic notes on western Canadian and Artic plants. Can. Jour. Bot. 42: 473-494.

Randhawa, A.S. and K.I. Beamish. 1972. The distribution of Saxifraga ferruginea and the problem of refugia in northwestern North America. Can. Jour. Bot. 50: 70-87.

Ridley, H.N. 1930. The Dispersal of Plants throughout the World. Ashford.

Russell, N.H. 1955. Local introgression between Viola cucullata Ait. and V. septentrionalis Greene. Evolution 9: 436-444.

Russell, N.H. and M. Cooperrider. 1955. Prediction of an introgressant in Viola. Amer. Midl. Nat. 54: 42-51.

Smith, H.H. and K. Daly. 1959. Discrete populations derived by intraspecific hybridization and selection in Nicotiana. Evol. 13: 476-487.

Smith, I. (editor) 1969. Chromatographic and Electrophoretic Techniques. Third edition. Vol. I. W. Heineman.

Stafleu, F.A., C.E.B. Bonner, R. McVaugh, R.D. Meikle, R.C. Rollins, R. Ross, J.M. Schopf, G.M. Schulze, R. DeVilmorin. 1972. International Code of Botanical Nomenclature. Oosthoek's Uitgeversmaatschappij, Utrecht.

Stebbins, G.L. 1950. Variation and Evolution in Plants. Columbia University Press, N.Y.

Stebbins, G.L. 1971. Chromosomal Evolution in Higher Plants. Edward Arnold Ltd., London.

Stebbins, G.L., B.L. Harvey, E.L. Cox, J.N. Rutger, G. Jelencovic and E. Yagil. 1963. Identification of the ancestry of an amphiploid Viola with the aid of paper chromatography. Amer. Jour. Bot. 50: 830-839.

Strelkova, O. 1938. Polyploidy and geographo-systematic groups in the genus Alopecurus L. Cytologia 8: 468-480.

Taylor, R.T. and R.P. Brockman. 1966. Chromosome numbers of some western Canadian plants. Can. Jour. Bot. 44: 1093-1103.

Tischler, G. 1935. Die Bedeutung der Polyploidie fur die Verbreitung der Angiospermen. Bot. Jb. 67: 1-36.

Tjio, H.J. and A. Levan. 1950. The use of oxyquinoline in chromosome analysis. An. Aula Die 2: 21-64.

Valentine, D.H. 1950. The experimental taxonomy of two species of Viola. New Phytol. 49: 193-212.

Valentine, D.H. 1962. Variation and evolution in the genus Viola. Preslia 34: 190-206.

Valentine, D.H. and A. Love. 1958. Taxonomic and Biosystematic categories. Brittonia 10: 153-166.